Supporting Information

Identification of uric acid gluconucleoside-ascaroside conjugates in *Caenorhabditis elegans* by combining synthesis and MicroED

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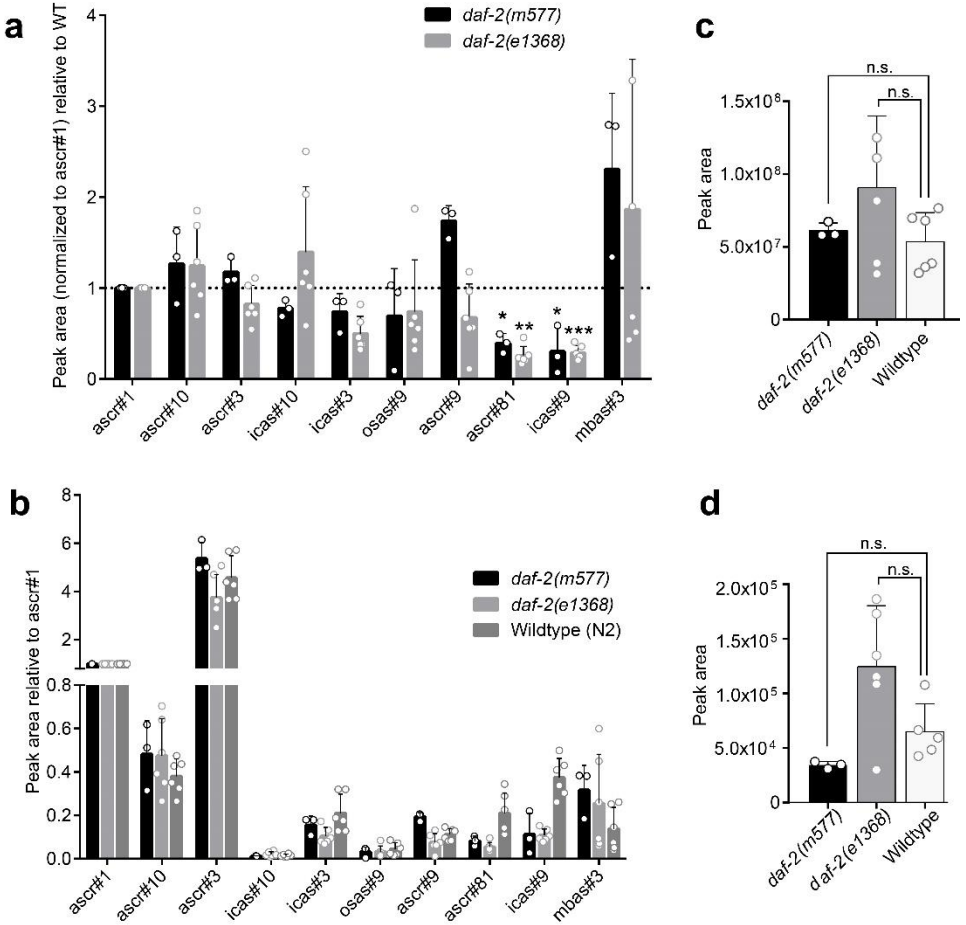


Figure S1. (a) Quantification of a panel of ascarosides, normalized to the abundance of ascr#1, in *C. elegans exo*-metabolome (media) samples of *daf-2(e1368)* and *daf-2(m577)* mutants, relative to wildtype (N2). (b) Quantification of a panel of ascarosides, normalized to the abundance of ascr#1, in *C. elegans exo*-metabolome (media) samples for *daf-2(e1368)* and *daf-2(m577)* mutants as well as wildtype (N2). (c) Quantitation of ascr#1 in *daf-2(e1368)*, *daf-2(m577)*, and wildtype (N2) *exo*-metabolome (media) samples. (d) Quantitation of gluric#1 (**13**) in *daf-2(e1368)*, *daf-2(m577)*, and wildtype (N2) *endo*-metabolome (pellet) samples. *p<0.05, **p<0.01, ***p<0.001, n.s., not significant.

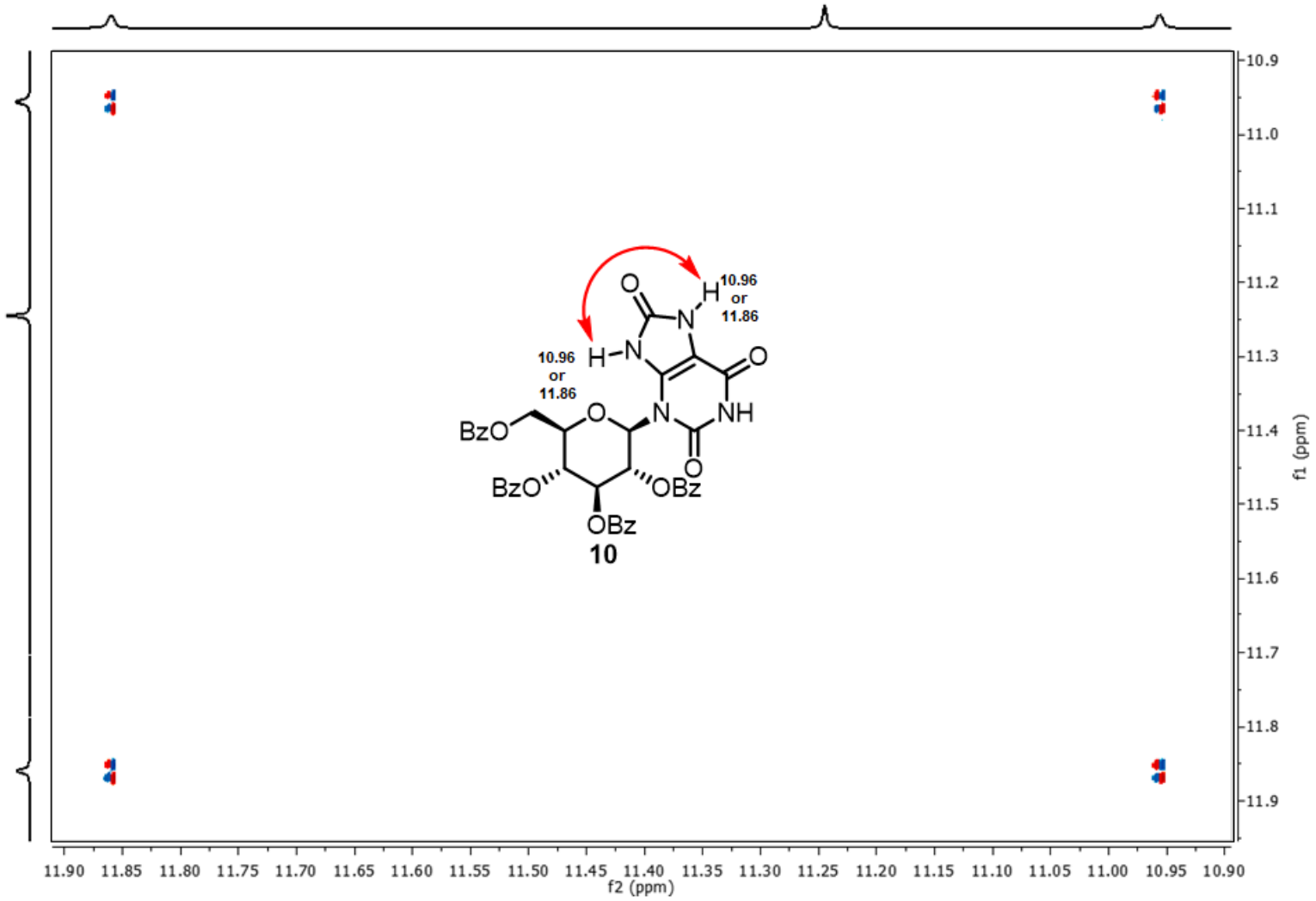


Figure S2. Section of dqfCOSY spectrum (800 MHz, DMSO-d_6) of **10** displaying long-range (^{1}H,^{1}H)-couplings of protons attached to the uric acid moiety.

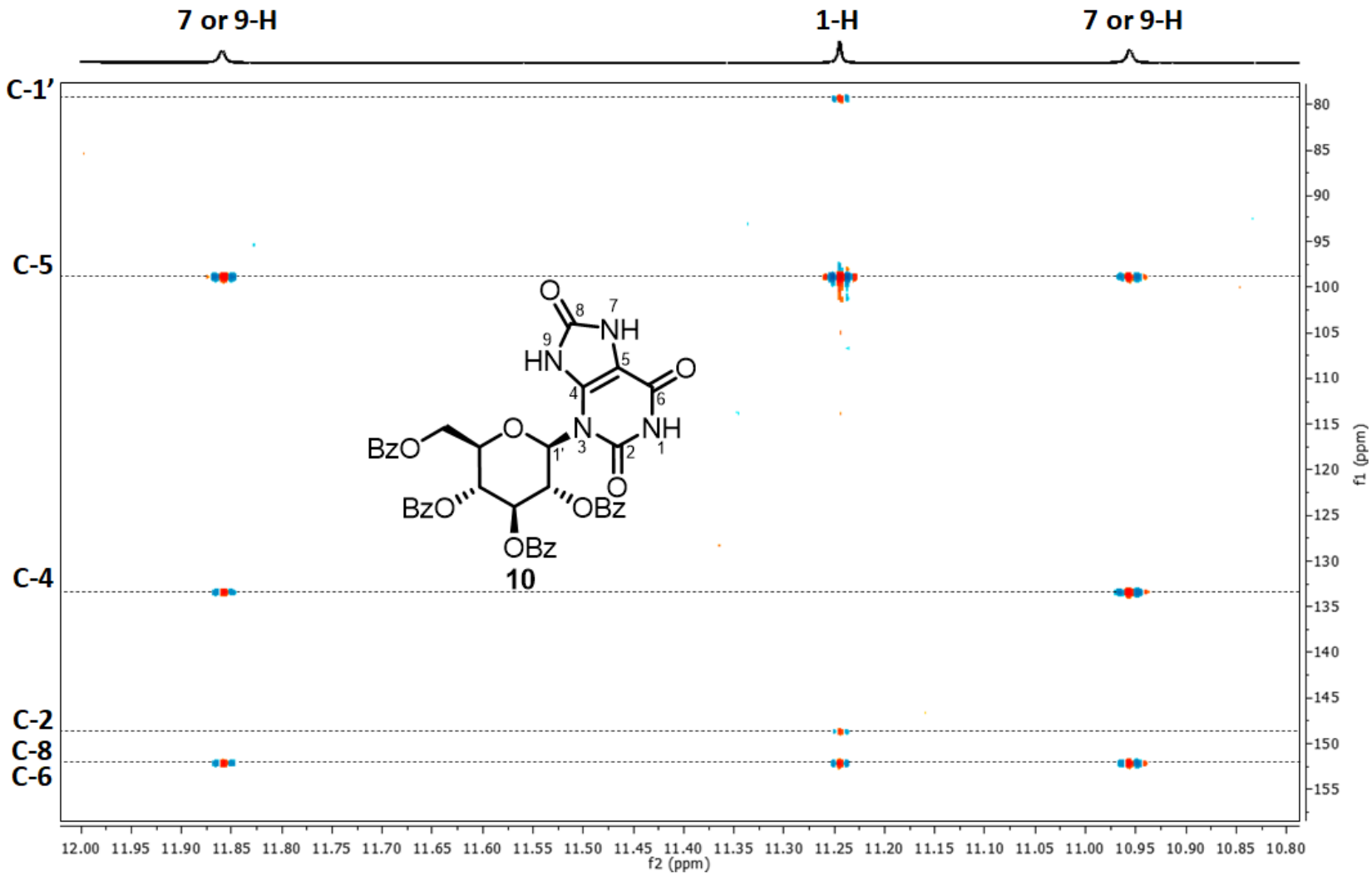


Figure S3. Section of HMBC spectrum (800 MHz, DMSO-d_6) of **10** showing signals for the protons attached to the uric acid moiety. Note that C-6 and C-8 chemical shifts are near identical.

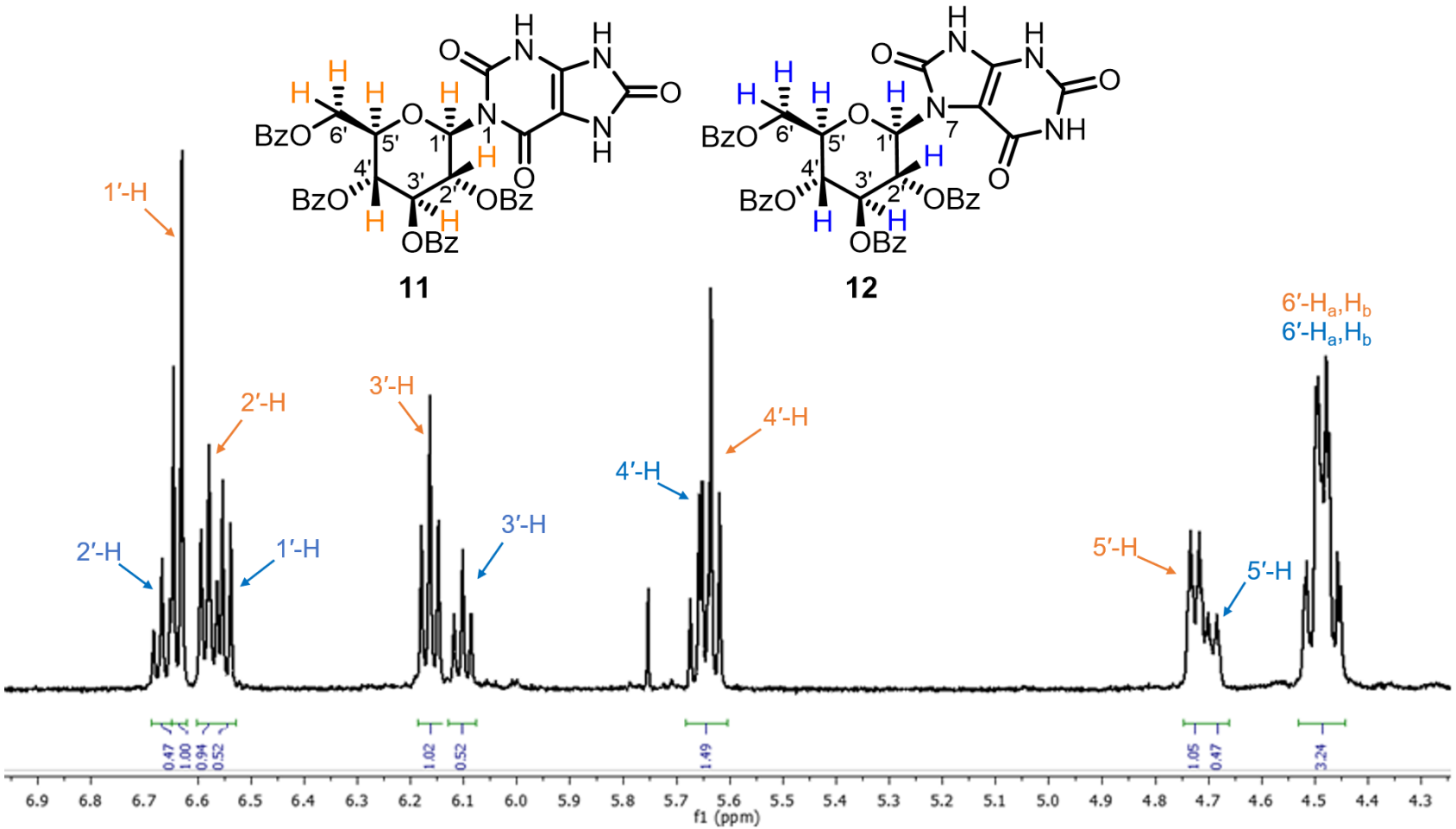
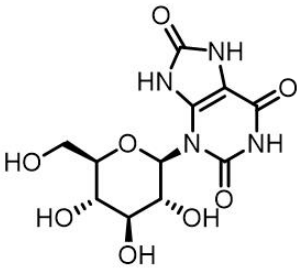


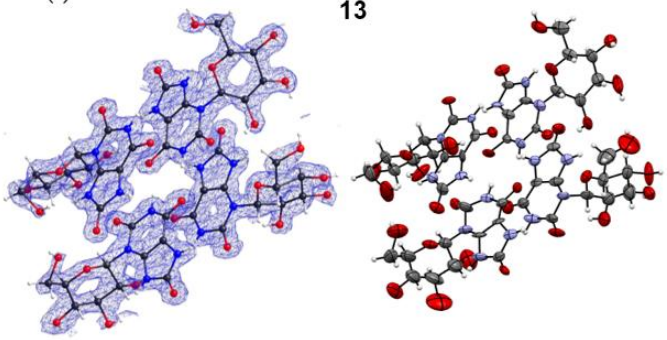
Figure S4. Section of ^{1}H NMR spectrum (800 MHz, DMSO-d_6) of a 2:1 mixture of minor regioisomers **11** and **12**, respectively. Orange labels represent N^1 regioisomer **11** and blue labels N^7 regioisomer **12**. See Tables S2 and S3 for full assignments.

a

Structure	Gluric#1 (**13**)
Stoichiometric formula	$C_{11}H_{14}N_4O_8$
Formula weight	330.25
Temperature	~100 K
Wavelength	0.025 Å
Crystal system	Triclinic
Space group	P1
Unit cell dimensions	a = 8.990(9) Å α = 93.05(9)° b = 9.730(2) Å β = 94.62(5)° c = 14.16(3) Å γ = 108.54(5)°
Resolution	1.0 Å
Observed reflections	8144
Unique reflections	2122
Completeness	87.1%
R_{obs}	15.5%
R_{meas}	17.7%
I/σI	5.79
$CC_{1/2}$	95.4%
R_1	0.1401
wR_2	0.3199
GooF	1.223



13



b

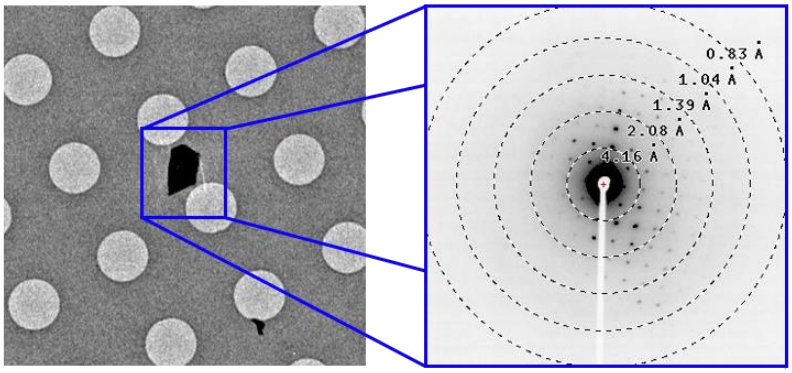


Figure S5. (a) MicroED data, statistics, and structures for **13**. (b) Example of an electron diffraction pattern obtained from **13**. Grid holes are 1 µm in diameter.

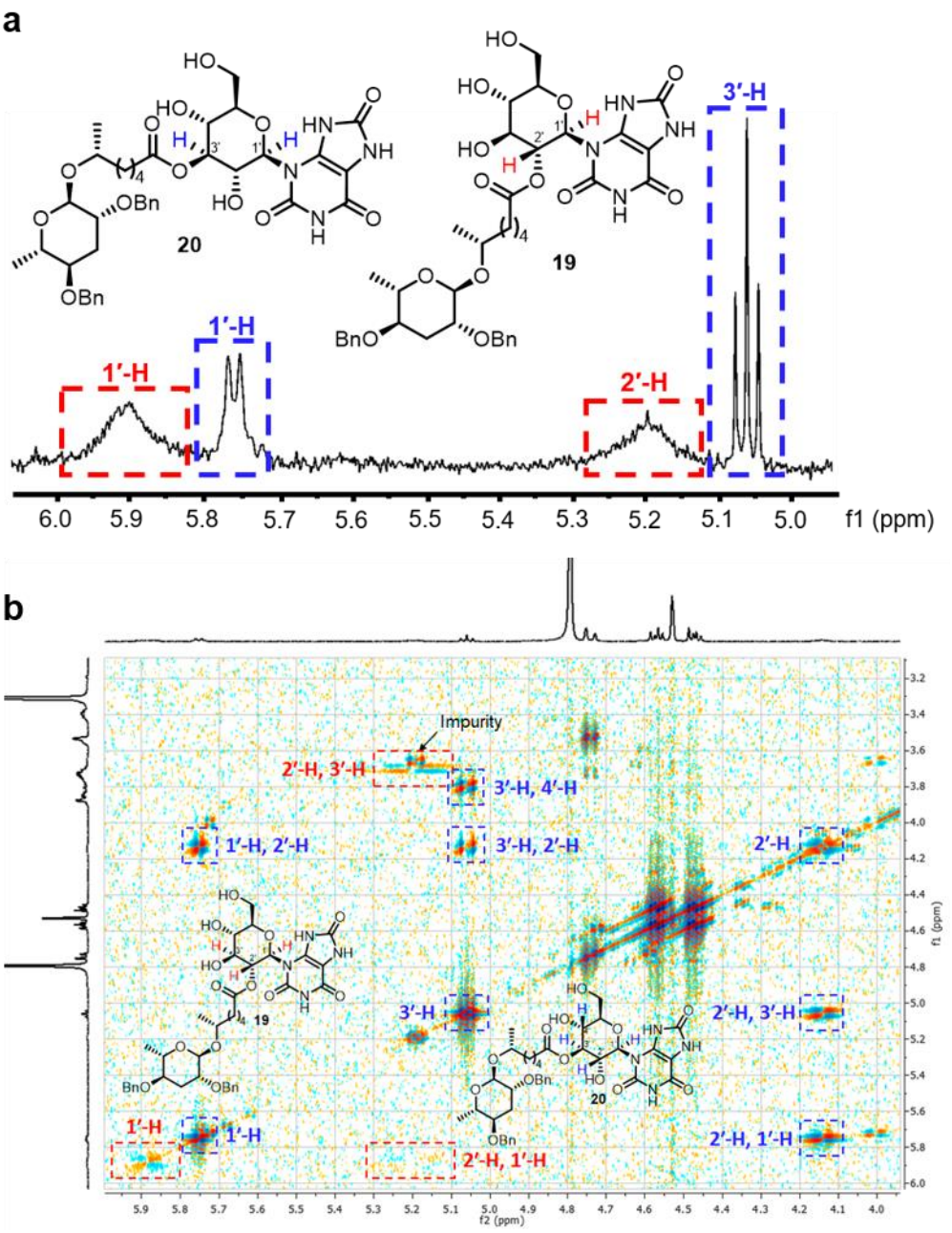


Figure S6. (a) Section of ^{1}H NMR spectrum (600 MHz, methanol-d_4) of a 2:1 mixture of minor regioisomers **19** and **20**, respectively. Signals of glucoside protons in **19** (boxed red) exhibit extreme line broadening, whereas signals for the glucoside protons in **20** (boxed blue) appear reasonably sharp. (b) Section of dqfCOSY spectrum (600 MHz, methanol-d_4) of a mixture of **19** and **20**, showing important cross-peaks of protons in the sugar moieties, some of which exhibit extreme line broadening. Spin system for 2'-*O* isomer (**19**) is shown boxed in red and 3'-*O* isomer (**20**) in blue. Cross-peaks are annotated as (f2, f1). See Tables S6 and S7 for full assignments.

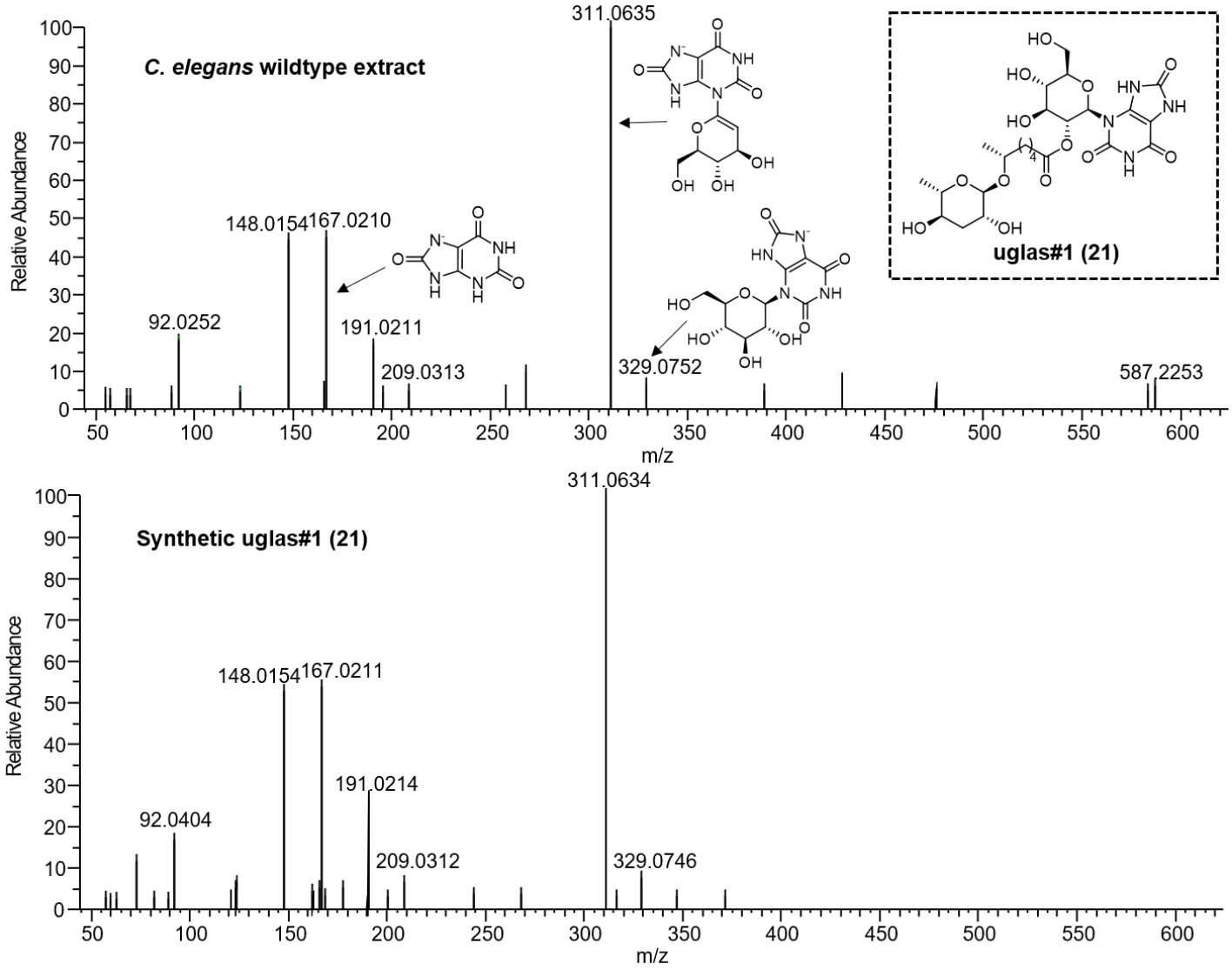


Figure S7. MS/MS spectra (ESI-) of uglas#1 (**21**) from *C. elegans* wildtype (N2) metabolome extract (top) and synthetic standard (bottom).

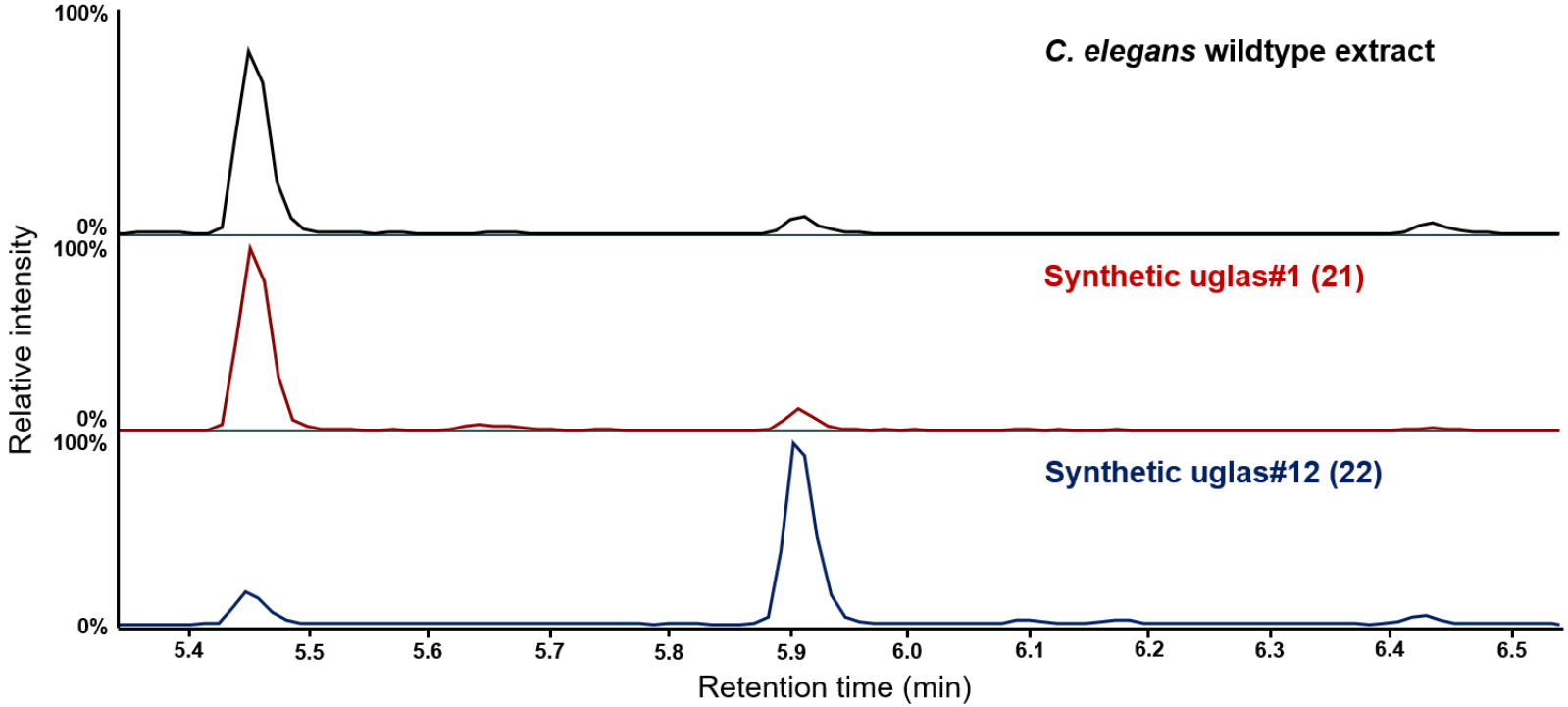


Figure S8. HPLC-HRMS (ESI-, using a C18 column) extracted ion chromatograms for *m/z* 587 of uglas#1 isomers from *C. elegans* wildtype (N2) *endo*-metabolome extract (top), synthetic uglas#1 (**21**) standard (middle), and synthetic uglas#12 (**22**) standard (bottom). Synthetic samples contain small amounts of other uglas#1 isomers. Late peak at 6.4 min. represents uglas#14 (**23**), see Figure 3b for further details.

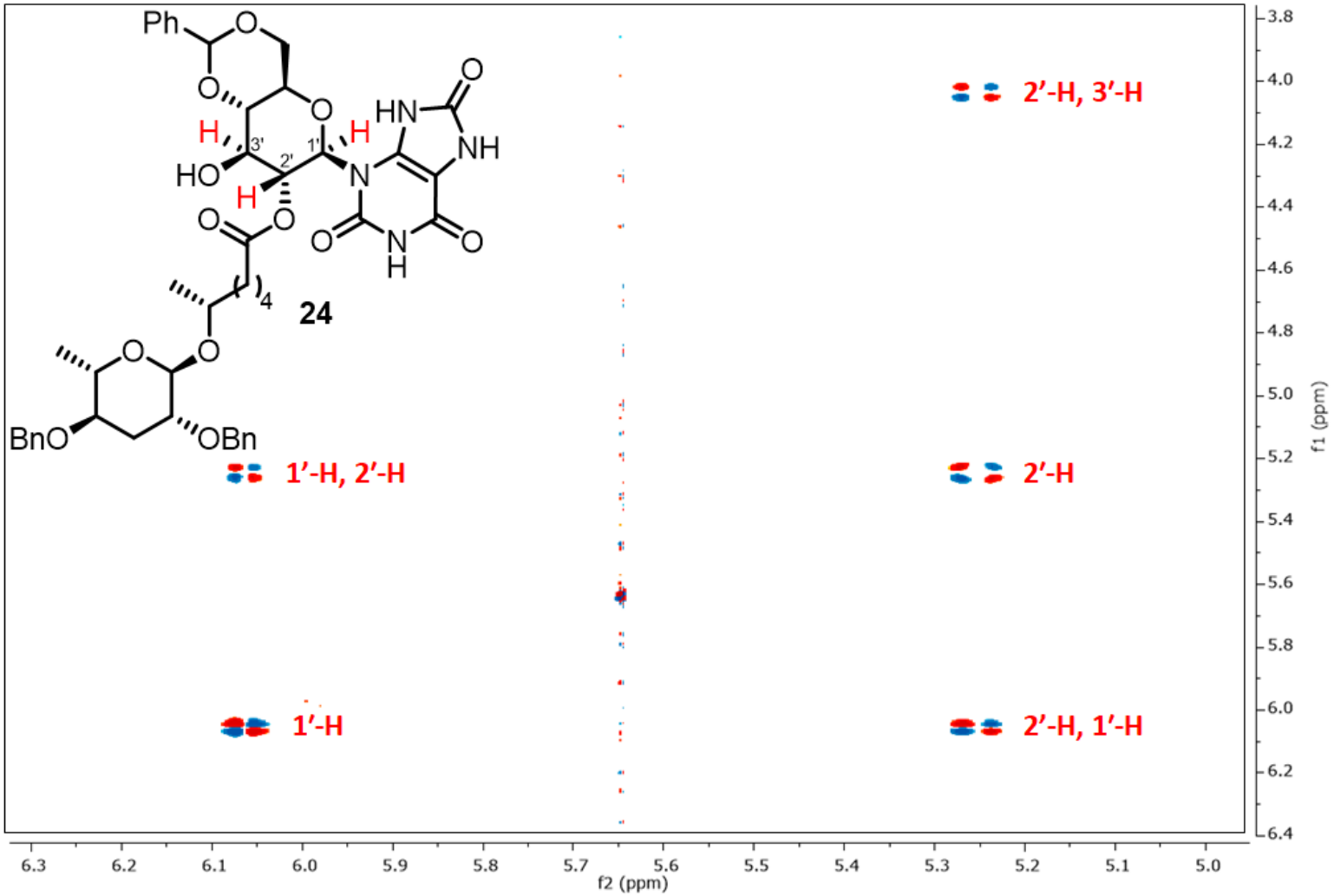


Figure S9. Section of dqfCOSY spectrum (600 MHz, methanol-d_4) of **24** showing cross-peaks that indicate 2'-*O* substitution. Cross-peaks are annotated as (f2, f1). See Table S11 for full assignments.

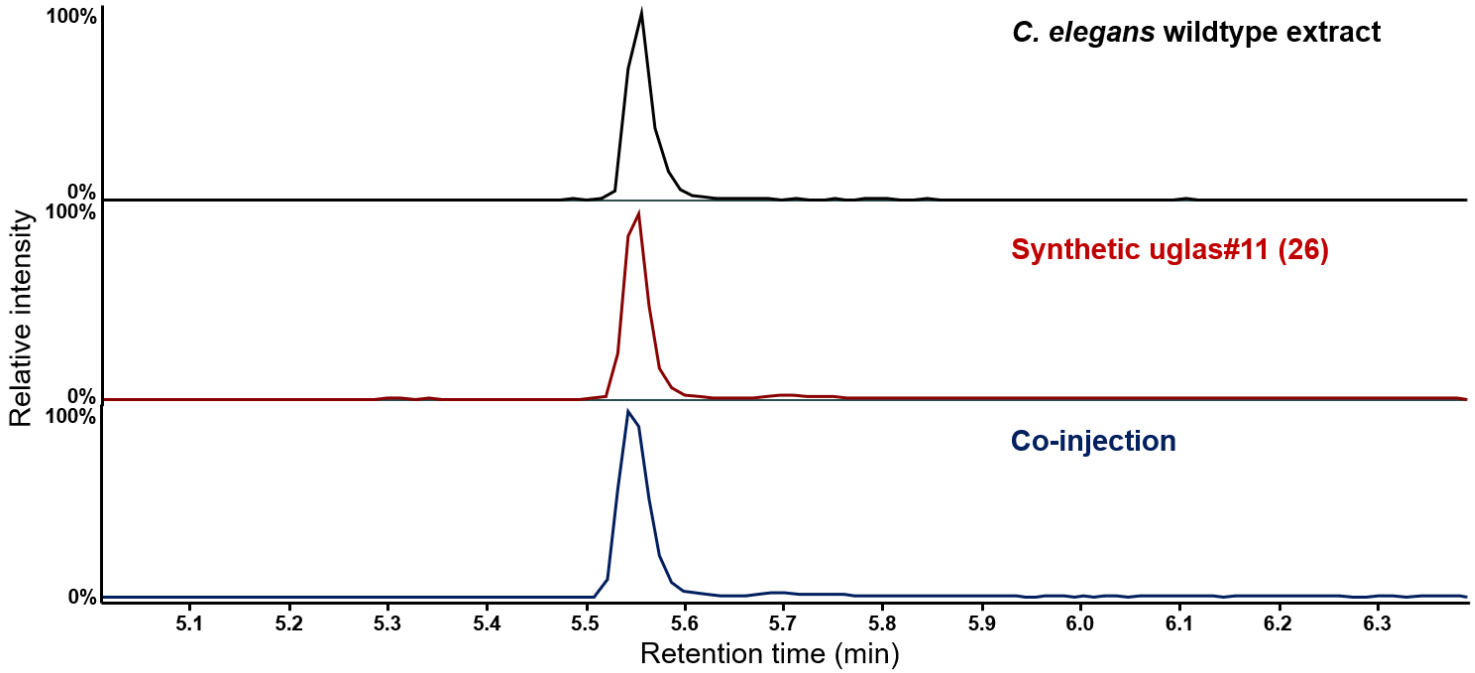


Figure S10. HPLC-HRMS (ESI-, using a C18 column) extracted ion chromatograms for *m/z* 667 of uglas#11 (**26**) from *C. elegans* wildtype (N2) *endo*-metabolome extract (top), synthetic standard (middle), and co-injection (bottom).

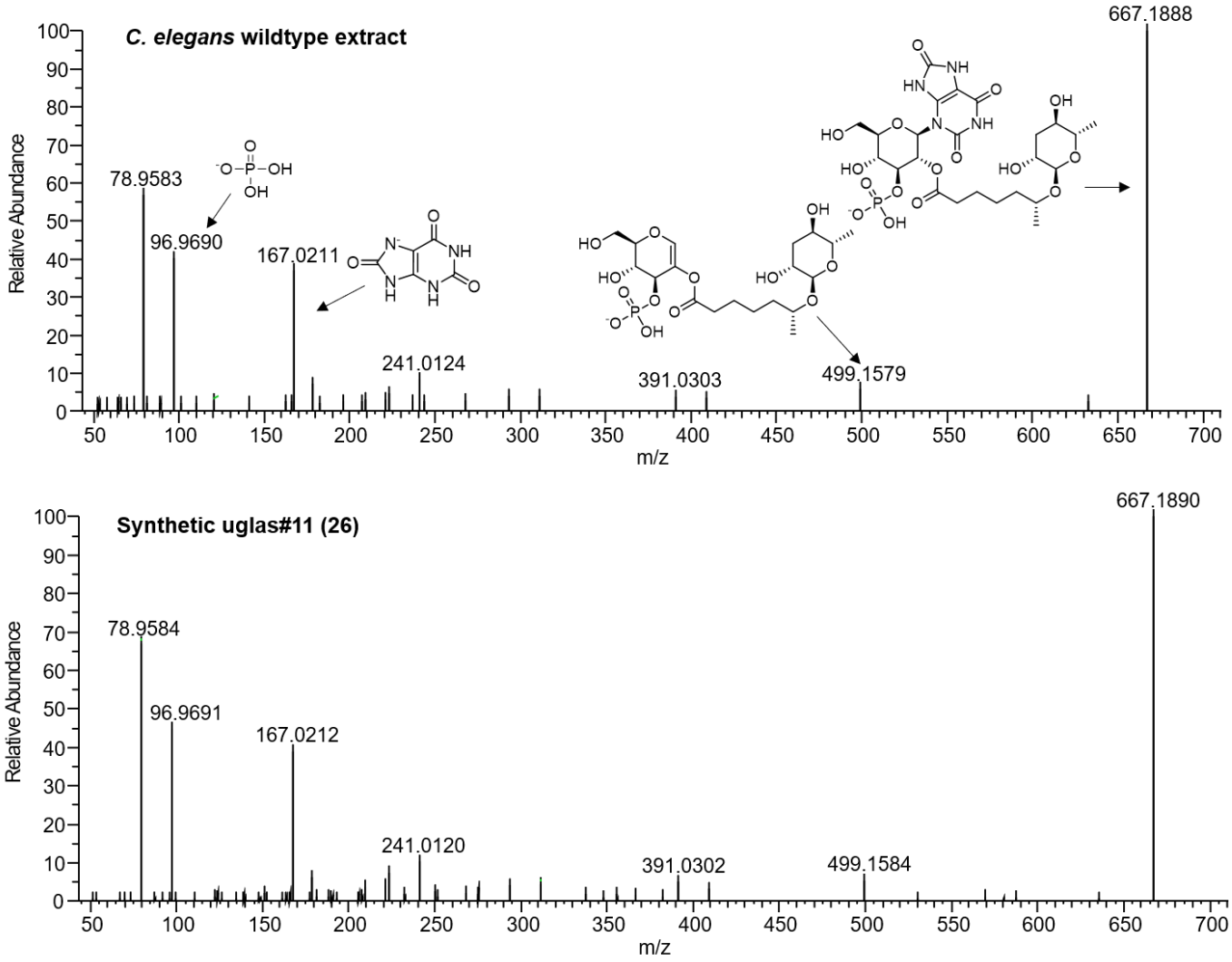


Figure S11. MS/MS spectra (ESI-) of uglas#11 (**26**) from *C. elegans* wildtype (N2) metabolome extract (top) and synthetic standard (bottom).

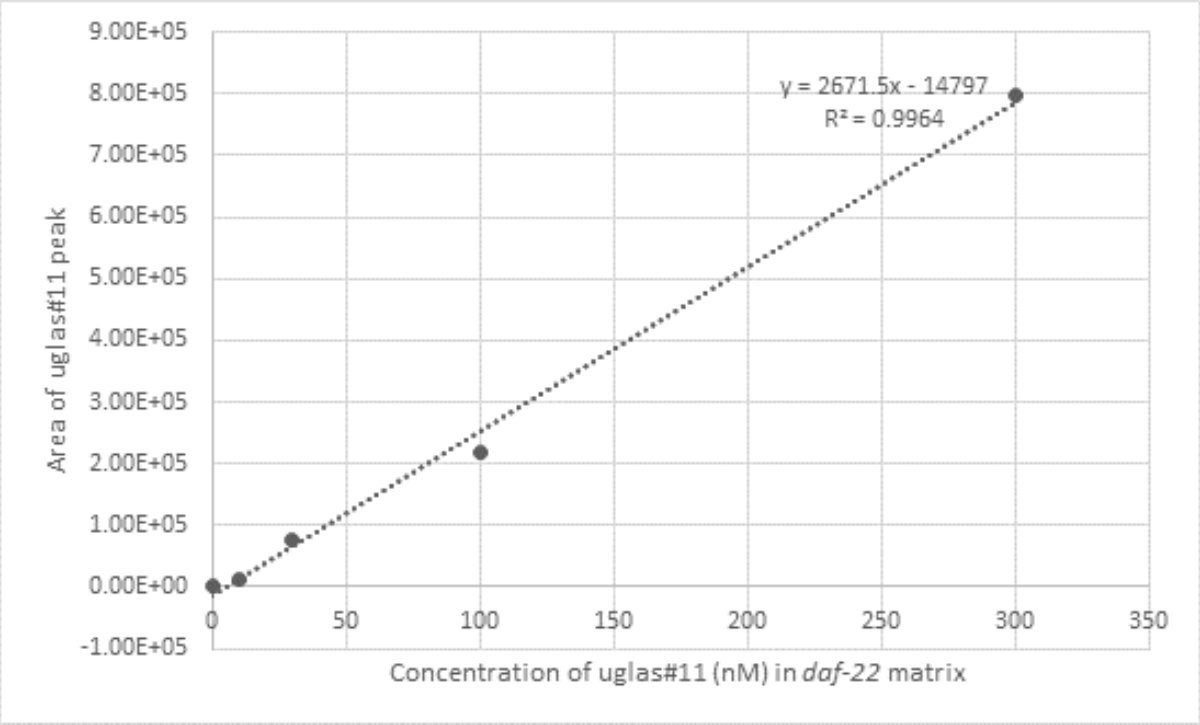


Figure S12. Standard curve for mass spectrometric quantitation of uglas#11 (**26**) in *C. elegans*, using synthetic uglas#11 spiked into a matrix of *daf-22* mutant metabolome. Peak areas of uglas#11 (**26**) were determined for five different concentrations and used to quantify the amount of uglas#11 in N2 and *daf-2* mutant samples.

2. Methods

Nematode strains

N2 (wildtype), RB859 [*daf-22(ok693)*], DR1572 [*daf-2(e1368)*], and DR1567 [*daf-2(m577)*] were provided by the Caenorhabditis Genetics Center (CGC), which is funded by the NIH Office of Research Infrastructure Programs (P40OD010440).

Nematode cultures

Cultures were started by chunking *C. elegans* onto 10 cm NGM plates (seeded with 750 µL of OP50 *E. coli* grown to stationary phase in Lennox Broth) and incubated at 22 °C. Once the food was consumed, each plate was washed into 25 mL of S-complete medium in 125 mL Erlenmeyer flask, supplemented with 1 mL of liquid OP50 *E. coli* (liquid OP50 food grown in Terrific Broth to stationary phase at 37 °C, pelleted and resuspended at 1 g per 1 mL M9 buffer). Liquid *C. elegans* cultures were incubated at 22 °C shaking at 220 RPM for 67 hr. Cultures were then pelleted at 1000 G 1 min 22 °C. The supernatant was discarded, and the pellet was washed with 30 mL H_2O. *C. elegans* were then pelleted as above, the supernatant was discarded, and to the pellet 24 mL H_2O, 6 mL bleach, and 900 µL 10 M NaOH and shaken for 3 min to lyse *C. elegans* bodies and isolate eggs. Samples were then pelleted again at 1000 G 1 min 22 °C, and washed twice with 30 mL M9 buffer, then resuspended with 5 mL of M9 buffer in 50 mL centrifuge tubes. Samples were placed on a rocker and shaken overnight at 22 °C to allow for L1 larvae to hatch. Hatched L1s were counted. 70,000 L1s were added to 25 mL of S-complete supplemented with 1 mL of liquid OP50 *E. coli* and incubated at 220 RPM and 22 °C in 125 mL Erlenmeyer flasks. After 70 hr, cultures were pelleted at 1000 G for 2 min at 22 °C. Supernatant was saved as *C. elegans* “media” and the worm bodies (“pellet”) was washed two times with M9 buffer. Separated media and worm pellet samples were flash frozen over liquid nitrogen and then lyophilized to dryness.

Metabolite extraction

Dried pellet samples were crushed and homogenized by shaking with 2.5 mm steel balls at 1300 RMP for 3 min in 30 s pulses while chilled with liquid nitrogen (SPEX sample prep miniG 1600). Thus powdered media and pellet samples were extracted with 15 mL methanol in 50 mL centrifuge tubes, rocking overnight at 22 °C. Extractions were pelleted at 5000 G for 10 min at 4 °C, and supernatants were transferred to 20 mL glass scintillation vials. Samples were then dried in a SpeedVac (Thermo Fisher Scientific) vacuum concentrator. Dried materials were resuspended in 1 mL methanol and vortexed for 1 min. Samples were pelleted at 5000 G for 5 min 22 °C, and supernatants were transferred to 2 mL HPLC vials and dried in a SpeedVac vacuum concentrator. Samples were then resuspended in 200 µL of methanol, transferred into 1.7 mL Eppendorf tubes, and centrifuged at 18,000 G for 20 min at 4 °C. Clarified extracts were transferred to fresh HPLC vials and stored at -20 °C until analysis.

Mass spectrometric analysis

High resolution LC-MS analysis was performed on a Thermo Fisher Scientific Vanquish Horizon UHPLC System coupled with a Thermo Q Exactive HF hybrid quadropole-orbitrap high-resolution mass spectrometer quipped with a HESI ion source. 1 µL of extract was injected and separated using at water-acetonitrile gradient on a Thermo Scientific Hypersil GOLD C18 column (150 mm x 2.1 mm 1.9 um particle size 175 Å pore size, Thermo Scientific) and maintained at 40 °C. Solvents were all purchased from Fisher Scientific as HPLC grade. Solvent A: 0.1% formic acid in water; solvent B: 0.1% formic acid in acetonitrile. A/B gradient started at 1% B for 5 min, then from 1% to 100% B over 20 min, 100% for 5 min, then down to 1% B for 3 min to equilibrate the column. Mass spectrometer parameters: 3.5 kV spray voltage, 380 °C capillary temperature, 300 °C probe heater temperature, 60 sheath flow rate, 20 auxiliary flow

rate, 1 spare gas ; S-lens RF level 50.0, resolution 240,000, *m/z* range 100-1200 *m/z*, AGC target 3e6. Instrument was calibrated with positive and negative ion calibration solutions (Thermo-Fisher) Pierce LTQ Velos ESI pos/neg calibration solutions. Peak areas were determined using Xcalibur 2.3 QualBrowser version 2.3.26 (Thermo Scientific) using a 5 ppm window around the *m/z* of interest. Concentrations of metabolites were calculated by dividing the peak area by the volume of the initial worm media (25 mL) or estimated volume of the worm pellet (~0.35 mL).

Quantification of uglas#11 in *C. elegans endo*-metabolome samples

The standard curve (Figure S12) was generated from dilution of pure uglas#11 (**26**) at five different concentrations (0 nM, 10 nM, 30 nM, 100 nM, 300 nM) in a *daf-22* metabolome matrix followed by MS analysis. Peak areas for *m/*z = 667.1869 were determined using Thermo Fisher Scientific Freestyle software and a linear equation was generated. Quantification of uglas#11 (**26**) in N2 assumed a per-worm volume of 0.0035 mm^3, yielding a total volume of 0.24 mL for the ~70,000 worms used in our experiments, consistent with worm pellet volumes of ~0.3 mL measured post-harvest following comtrifugation.[1] The concentration of uglas#11 in N2 *endo*-metabolome samples was determined from the average peak areas of two sets of biological replicates, each containing three technical replicates.

Purification via preparative HPLC

uglas#11 (**26**) was purified using a Thermo Fisher Scientific Vanquish Horizon UHPLC system and a Agilent Zorbax Eclipse XDB-C18 column (4.6 × 250 mm, 5 µm particle diameter), with flow rate of 1 mL/min at 25 °C. A solvent gradient scheme was used as follows: isocratic 1% acetonitrile (0.1 % formic acid) 99% H_2O (0.1 % formic acid) for 1 min, linear increase to 40% acetonitrile (0.1 % formic acid) 60% H_2O (0.1 % formic acid) over a 14 min period, linear increase to 90% acetonitrile (0.1 % formic acid) 10% H_2O (0.1 % formic acid) over a 9 min period, linear decrease to 1% acetonitrile (0.1 % formic acid) 99% H_2O (0.1 % formic acid) over a 1 min period, and isocratic 1% acetonitrile (0.1 % formic acid) 99% H_2O (0.1 % formic acid) for 5 min.

MicroED data and statistics of 13

Quantifoil holey-carbon EM grids were placed in a dram vial with purified **13** and shaken lightly. Residual compound was removed by tapping lightly against the surface of a filter paper. All diffraction data was collected on FEI Tecnai F200C electron microscope with an operating voltage of 200 keV, corresponding to a wavelength of 0.025 Å, using Gatan 626 cryo-holder under cryogenic temperature (100 K). During data acquisition, the crystal of interest was isolated using a selected area aperture and continuously rotated at a rate of -0.3º/s over a tilt range of 50‑100º. Continuous rotation diffraction data was recorded using rolling shutter mode with a Ceta-D CMOS 4k x 4k camera, integrating at a rate of 3 s per frame and binning by 2 to produce final images of 2k x 2k.[2]

Diffraction movies saved as SER files were converted to SMV format using ser2smv software as described previously.[3] Frames were indexed and integrated in XDS.[4] Data from four crystals were scaled and merged together using XSCALE[5] to produce the final data set. Finally, intensities were converted to SHELX format using XDSCONV.[5]

The structure of **13** was solved *ab initio* using direct methods in SHELXD[6] and refined with SHELXL[7] in ShelXle.[8] All non-hydrogen atoms were refined anisotropically, and hydrogen atoms were placed using the riding model.

Crystallographic information files (CIF) for compound **13** have been deposited at the Cambridge Crystallographic Data Center (Deposition Number: 2020283).

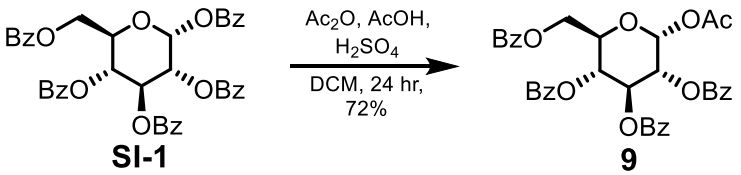
General synthetic methods

All oxygen and moisture-sensitive reactions were carried out under argon (Ar) atmosphere in flame-dried glassware. Solutions and solvents sensitive to moisture and oxygen were transferred via standard syringe and cannula techniques. Trimethylsilyl trifluoromethanesulfonate (TMSOTf) and dibenzyl *N*,*N*-diethylphosphoramidite were transferred to Schlenk flasks prior to use. Unless stated otherwise, all chemicals and reagents used for synthetic compound preparation were purchased from Sigma-Aldrich. *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC•HCl) was purchased from AMRESCO. Methanolic ammonia (7N) was purchased from Acros Organics. Acetic acid (AcOH), acetonitrile (ACN), dichloromethane (DCM), ethyl acetate (EtOAc), formic acid, hexanes, hydrochloric acid (HCl), methanol (MeOH), and sulfuric acid (H_2SO_4) used for chromatography and as a reagent or solvent were purchased from Fisher Scientific. Acetonitrile (ACN), 1,2-dichloroethane (DCE), dichloromethane (DCM), and *N*,*N*-dimethylformamide (DMF) were dried with 3Å molecular sieves prior to use. Thin-layer chromatography (TLC) was performed using J. T. Baker Silica Gel IB2F plates. Flash chromatography was performed using Teledyne Isco CombiFlash systems and Teledyne Isco RediSep Rf silica and C18 columns. All deuterated solvents were purchased from Cambridge Isotopes. Nuclear Magnetic Resonance (NMR) spectra were recorded on Varian INOVA 600 (600 MHz) spectrometer at Cornell University's NMR facility and Bruker AVANCE III HD 800 MHz (800 MHz) or Bruker AVANCE III HD 600 MHz (600 MHz) at SUNY ESF's NMR facility. ^{1}H NMR chemical shifts are reported in ppm (δ) relative to residual solvent peaks (7.26 ppm for chloroform-*d*, 3.31 ppm for methanol-d_4, 2.50 for DMSO-d_6). NMR-spectroscopic data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constants (Hz), and integration and often tabulated including 2D NMR data. ^{13}C NMR chemical shifts are reported in ppm (δ) relative to residual solvent peaks (77.16 ppm for chloroform-*d*, 49.00 ppm for methanol-d_4, 39.52 for DMSO-d_6). All NMR data processing was done using MNOVA 12.0.1 (https://mestrelab.com/).

3. Synthetic procedures

3.1. Synthesis of gluric#1 (13)

1-*O*-Acetyl-2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranose (9)

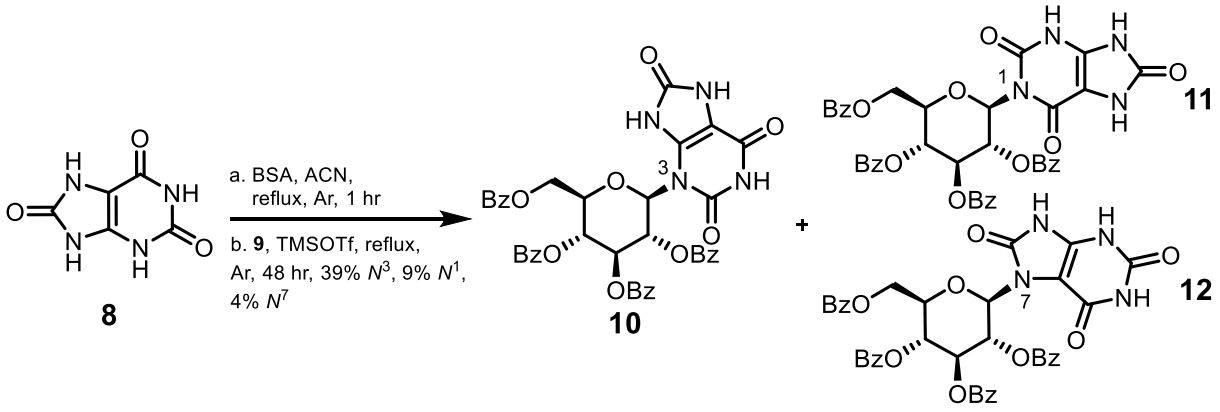


To 40 mL of DCM was added 1,2,3,4,6-penta-*O*-benzoyl-α-D-glucopyranose (**SI-1**, 6.56 g, 9.38 mmol, 1.0 equiv.), acetic anhydride (17.68 mL, 187 mmol, 20.0 equiv.), AcOH (8.04 mL, 141 mmol, 15.0 equiv.), and H_2SO_4 (1.50 mL, 28.1 mmol, 3.0 equiv.). The reaction mixture was stirred at room temp. for 24 hr, diluted with DCM, then quenched with the addition of sat. aq. sodium bicarbonate ($NaHCO_3$). The organics were washed three times with sat. aq. $NaHCO_3$, dried with magnesium sulfate (Mg_2SO_4), and concentrated *in vacuo*. Flash chromatography on silica using a gradient of 20-40% EtOAc in hexanes afforded 1-*O*-acetyl-2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranose (**9**, 4.30 g, 72%) as a white solid. Additional purification may be done by recrystallization from hexanes/EtOAc mixtures.

^{1}H NMR (600 MHz, chloroform-*d*): δ (ppm) 8.03 (d, *J* = 7.8 Hz, 2H), 7.96 – 7.90 (m, 4H), 7.87 (d, *J* = 7.8 Hz, 2H), 7.56 (t, *J* = 7.5 Hz, 1H), 7.54 – 7.49 (m, 2H), 7.47 – 7.41 (m, 3H), 7.38 (t, *J* = 7.8 Hz, 2H), 7.36 (t, *J* = 7.8 Hz, 2H), 7.30 (t, *J* = 7.8 Hz, 2H), 6.63 (d, *J* = 3.7 Hz, 1H), 6.17 (t, *J* = 10.0 Hz, 1H), 5.79 (t, *J* = 10.0 Hz, 1H), 5.54 (dd, *J* = 10.0, 3.8 Hz, 1H), 4.62 (dd, *J* = 12.2, 2.9 Hz, 1H), 4.52 (ddd, *J* = 10.2, 4.3, 2.9 Hz, 1H), 4.46 (dd, *J* = 12.2, 4.4 Hz, 1H), 2.23 (s, 3H). ^{1}H NMR spectroscopic assignments are similar to those previously reported.[9]

HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{36}H_{30}O_{11}Na$ 661.1680; found 661.1677.

Perbenzoylated glucosyluric acid isomers (10-12)



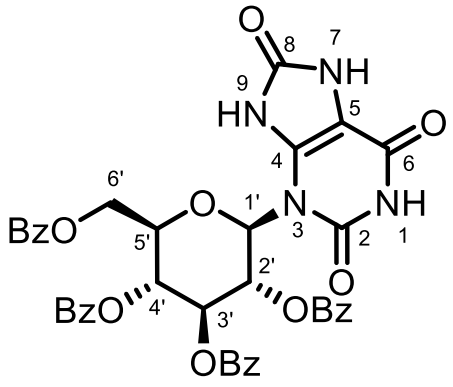
Under Ar, a mixture of *N*,*O*-bis(trimethylsilyl)acetamide (BSA) (1.0 mL, 4.1 mmol, 9.3 equiv.), uric acid (**8**, 147 mg, 0.88 mmol, 2.0 equiv.), and 4 mL of dry acetonitrile (ACN) was refluxed for 1 hr using a mineral oil bath. The resulting homogeneous solution was cooled to room temp., 1-*O*-acetyl-2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranose (**9**, 280 mg, 0.44 mmol, 1.0 equiv.) in 1 mL ACN and TMSOTf (0.25 mL, 1.38 mmol, 3.1 equiv.) were added, and the solution was refluxed

under Ar for 48 hr. The reaction mixture was diluted with EtOAc and quenched with cold sat. aq. $NaHCO_3$. Precipitated uric acid was allowed to settle to the bottom aqueous layer. The organics were extracted three times with EtOAc, dried with $MgSO_4$, filtered, and concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 0-50% MeOH in EtOAc furnished the perbenzoylated N^3 uric acid glucoside (**10**, 132 mg, 39%) as a white foam, separable from combined perbenzoylated N^1 (**11**, 30 mg, 9%) and N^7 uric acid glucosides (**12**, 15 mg, 4%) as off-white solids, and recovered **9** (105 mg, 37%). Note that N^3 uric acid glucoside (**10**) elutes around 5% MeOH, whereas combined perbenzoylated N^1 (**11**) and N^7 uric acid glucosides (**12**) around 35% MeOH.

HRMS (ESI) *m/z*: $[M - H]^-$ calcd for $C_{39}H_{29}O_{12}N_4$ 745.1787; found 745.1783 for all isomers.

See Tables S1–3 for NMR spectroscopic assignments of **10-12**.[10]

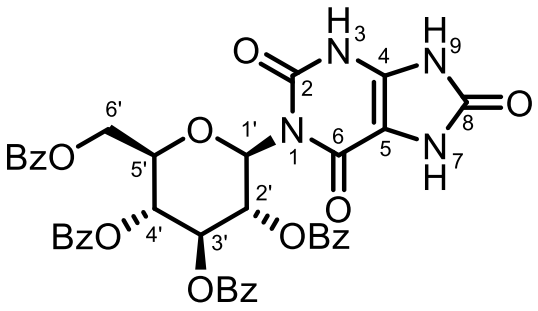
Table S1. NMR spectroscopic data for perbenzoylated N^3-(*β*-glucopyranosyl)uric acid (10). ^{1}H (800 MHz), HSQC, and HMBC NMR spectroscopic data were acquired in DMSO-d_6. Chemical shifts were referenced to δ((C**H**D$_2$)$_2$SO) = 2.50 ppm and δ((**^{13}C**HD$_2$)$_2$SO) = 39.52 ppm. ^{1}H NMR data for aromatic protons: δ 7.94 (d, *J* = 7.7 Hz, 2H), 7.86 (d, *J* = 7.8 Hz, 2H), 7.78 (d, *J* = 7.8 Hz, 2H), 7.74 (d, *J* = 7.9 Hz, 2H), 7.65 – 7.59 (m, 3H), 7.56 (t, *J* = 7.5 Hz, 1H), 7.49 – 7.44 (m, 6H), 7.41 (t, *J* = 7.6 Hz, 2H). ^{13}C NMR chemical shift data for aromatic and ester carbons: δ 165.4, 165.0, 164.9, 164.8, 134.0, 133.7, 133.3, 129.4, 129.2, 129.1, 128.8, 128.6, 128.4, 127.4.



10

Position	^{13}C [ppm]	^{1}H [ppm]	$J_{H,H}$ couplings [Hz]	HMBC correlations
1	-	11.25	-	C-2 (weak), C-5, C-6, C-1' (weak)
2	148.7	-	-	-
3	-	-	-	-
4	133.5	-	-	-
5	99.0	-	-	-
6	152.2	-	-	-
7	-	10.96 or 11.86	$J_{7,9}$ < 2	C-4, C-5, C-8
8	152.2	-	-	-
9	-	10.96 or 11.86	-	C-4, C-5, C-8
1'	79.5	6.69	$J_{1',2'}$ = 9.5	C-2, C-4, C-2', C-3', C-5'
2'	69.1	5.99	$J_{2',3'}$ = 9.5	C-1', C-3'
3'	73.3	6.27	$J_{3',4'}$ = 9.5	C-1', C-2', C-4', C-5'
4'	68.4	6.34	$J_{4',5'}$ = 9.5	C-3', C-5', C-6'
5'	74.2	4.84	$J_{5',6'a}$ = 3.6, $J_{5',6'b}$ = 5.6	C-1', C-3', C-4', C-6'
6'	63.0	4.51 (a), 4.57 (b)	$J_{6'a,6'b}$ = 12.2	C-4', C-5'

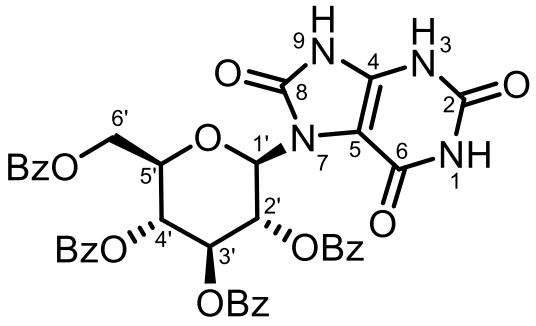
Table S2. NMR spectroscopic data for perbenzoylated N^1-(*β*-glucopyranosyl)uric acid (11). ^{1}H (800 MHz), HSQC, and HMBC NMR spectroscopic data were acquired in DMSO-d_6. Chemical shifts were referenced to δ((C**H**D$_2$)$_2$SO) = 2.50 ppm and δ((**^{13}C**HD$_2$)$_2$SO) = 39.52 ppm. ^{1}H NMR data for uric acid and aromatic protons: δ 10.56 (br s, 1H), 7.97 (d, *J* = 7.6 Hz, 2H), 7.83 (d, *J* = 7.9 Hz, 2H), 7.79 – 7.74 (m, 2H), 7.73 – 7.70 (m, 2H), 7.68 (m, 1H), 7.60 (t, *J* = 7.4 Hz, 2H), 7.57 – 7.52 (m, 3H), 7.48 – 7.42 (m, 4H), 7.39 (t, *J* = 7.6 Hz, 2H). ^{13}C NMR chemical shift data for aromatic and ester carbons: δ 165.2, 164.9, 164.8, 164.7, 133.7, 133.6, 133.5, 129.2, 129.1, 128.6.



11

Position	^{13}C [ppm]	^{1}H [ppm]	$J_{H,H}$ couplings [Hz]	HMBC correlations
1	-	-	-	-
2	148.9	-	-	-
3	-	-	-	-
4		-	-	-
5	-	-	-	-
6	151.8	-	-	-
7	-	-	-	-
8		-	-	-
9	-	-	-	-
1'	77.5	6.64	$J_{1',2'}$ = 9.2	C-2, C-6, C-2', C-3', C-5'
2'	68.7	6.58	$J_{2',3'}$ = 9.2	C-1', C-3'
3'	73.9	6.16	$J_{3',4'}$ = 9.4	C-2', C-4'
4'	68.2	5.64	$J_{4',5'}$ = 9.7	C-3', C-5', C-6'
5'	72.6	4.73	$J_{5',6'a}$ = 3.3, $J_{5',6'b}$ = 3.3	C-1', C-3', C-4'
6'	62.1	4.47 (a), 4.51 (b)	$J_{6'a,6'b}$ = 12.3	C-4', C-5'

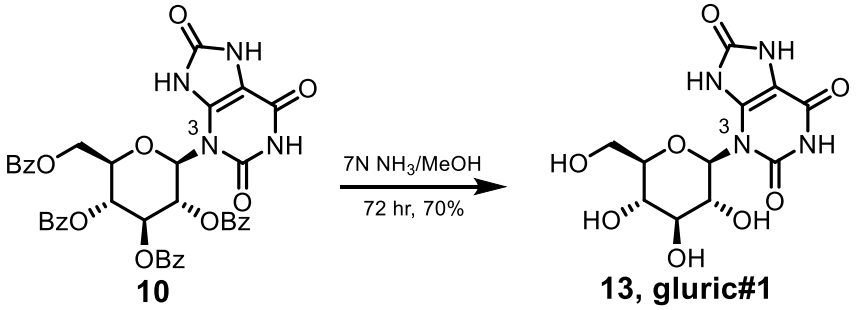
Table S3. NMR spectroscopic data for perbenzoylated N^7-(*β*-glucopyranosyl)uric acid (12). ^{1}H (800 MHz), HSQC, and HMBC NMR spectroscopic data were acquired in DMSO-d_6. Chemical shifts were referenced to δ((C**H**D$_2$)$_2$SO) = 2.50 ppm and δ((**^{13}C**HD$_2$)$_2$SO) = 39.52 ppm. ^{1}H NMR data for uric acid and aromatic protons δ 10.62 (br s, 1H), 7.97 (d, *J* = 7.6 Hz, 2H), 7.83 (d, *J* = 7.9 Hz, 2H), 7.79 – 7.74 (m, 2H), 7.73 – 7.70 (m, 2H), 7.68 (m, 1H), 7.60 (t, *J* = 7.4 Hz, 2H), 7.57 – 7.52 (m, 3H), 7.48 – 7.42 (m, 4H), 7.39 (t, *J* = 7.6 Hz, 2H). ^{13}C NMR chemical shift data for aromatic and ester carbons: δ 165.2, 165.0, 164.8, 164.5, 133.7, 133.6, 133.5, 129.2, 129.1, 128.6.



12

Position	^{13}C [ppm]	^{1}H [ppm]	$J_{H,H}$ couplings [Hz]	HMBC correlations
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	97.0	-	-	-
6	150.8	-	-	-
7	-	-	-	-
8	151.9	-	-	-
9	-	-	-	-
1'	80.1	6.55	$J_{1',2'}$ = 9.2	C-5, C-6, C-8, C-2', C-3', C-5'
2'	69.1	6.67	$J_{2',3'}$ = 9.3	C-1', C-3'
3'	74.0	6.11	$J_{3',4'}$ = 9.5	C-2', C-4'
4'	68.3	5.66	$J_{4',5'}$ = 9.6	C-3', C-5', C-6'
5'	72.7	4.69	$J_{5',6'a}$ = 3.3, $J_{5',6'b}$ = 3.3	C-1', C-3', C-4'
6'	62.1	4.47 (a), 4.51 (b)	$J_{6'a,6'b}$ = 12.3	C-4', C-5'

N^3-(*β*-glucopyranosyl)uric acid, gluric#1 (13)



10 **13, gluric#1**

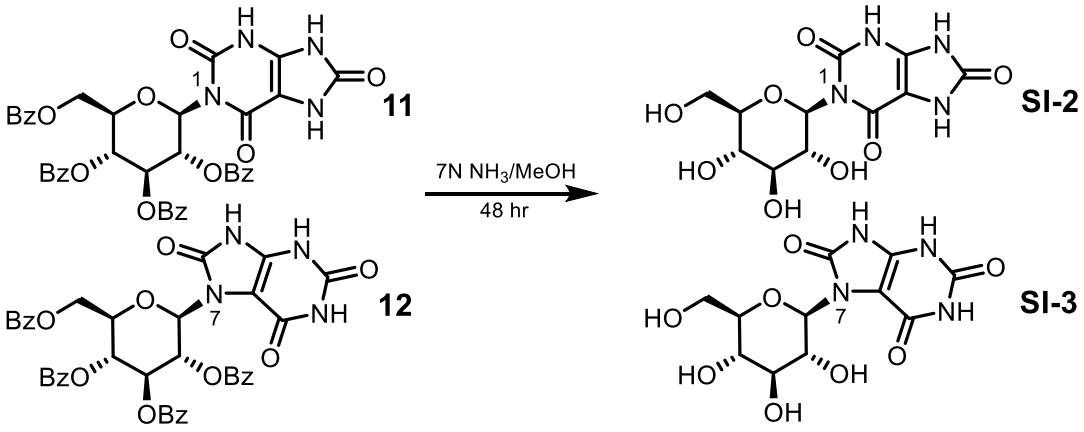
To a container was added 11.5 mL (80.5 mmol, 134 equiv.) of 7N methanolic ammonia and 450 mg (0.60 mmol, 1.0 equiv.) of perbenzoylated N^3 uric acid glucoside (**10**). The container was sealed, the reaction mixture was stirred at room temp. for 72 hr forming a white precipitate, and then concentrated *in vacuo*. The crude mixture was dissolved in water and washed three times with chloroform. The aqueous layer was concentrated until roughly 3 mL of solution remained and was then acidified to pH = 1 using conc. aq. HCl. Reversed phase flash column chromatography on C18 using a gradient of 0-10% MeOH (with 0.1% AcOH) in water (with 0.1% AcOH) afforded gluric#1 (**13**, 136 mg, 70%) as a white solid. A sample of gluric#1 was compared to those of isomers in *C. elegans* wildtype (N2) *exo*-metabolome samples by HPLC-HRMS on C18 (see Figure 2b).

^{1}H NMR (600 MHz, DMSO-d_6): δ 11.24 (br s, 1H), 11.12 (s, 1H), 10.83 (s, 1H), 5.42 (br s, 1H), 3.70 (dd, *J* = 11.9, 1.9 Hz, 1H), 3.53 (dd, *J* = 12.0, 6.7 Hz, 1H), 3.50-3.34 (m, 2H), 3.31 (ddd, *J* = 9.3, 6.7, 1.9 Hz, 1H), 3.23 (t, *J* = 8.8 Hz, 1H). Note: proton signal broadening was variable between samples.

^{13}C NMR (151 MHz, DMSO-d_6): δ 152.7, 152.4, 149.2, 135.0 (broad), 98.7, 83.3 (broad), 81.1, 77.2, 69.1, 68.9, 61.1.

HRMS (ESI) *m/z*: $[M - H]^-$ calcd for $C_{11}H_{13}O_8N_4$ $[M-H]^-$ 329.0739; found 329.0737.

Glucosyluric acid isomers (SI-2 – SI-3)



11 **12** **SI-2** **SI-3**

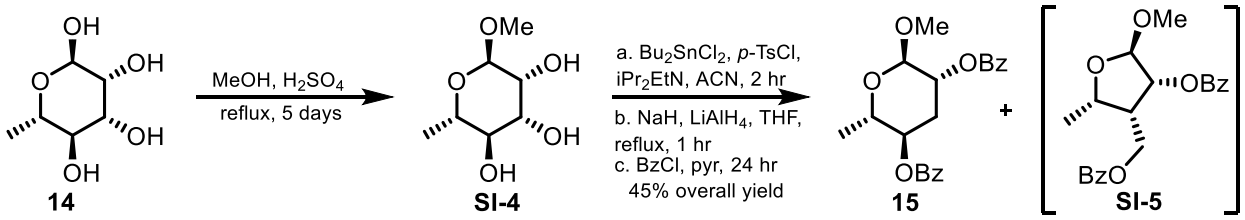
To a container was added 0.4 mL (2.8 mmol, 215 equiv.) of 7N methanolic ammonia and 10 mg (0.013 mmol, 1.0 equiv.) of a 2:1 mixture of perbenzoylated N^1 (**11**) and N^7 (**12**) uric acid glucosides, respectively. The container was sealed, the reaction mixture was stirred at room

temp. for 48 hr, and then concentrated *in vacuo*. The crude isomeric mixture of **SI-2** and **SI-3** was compared to those of isomers in *C. elegans* wildtype (N2) *exo*-metabolome samples by HPLC-HRMS on C18 (see Figure 2b).

HRMS (ESI) *m/z*: $[M - H]^-$ calcd for $C_{11}H_{13}O_8N_4$ $[M-H]^-$ 329.0739; found 329.0749 for both isomers.

3.2. Improved synthesis of dibenzoylated ascarylose 16

2,4-Di-*O*-benzoyl-1-*O*-methyl ascarylose (15)



Based on previously reported procedures[11–13], L-rhamnose·H_2O (**14**, 10.0 g, 55.0 mmol, 1.0 equiv.) was dissolved in MeOH (70 mL, 1.7 mol, 31.0 equiv.), H_2SO_4 (99%, 0.5 mL, 9.4 mmol, 0.2 equiv.) was added, and the resulting solution was heated at reflux for 5 days using a mineral oil bath. The reaction mixture was cooled to room temp. and concentrated *in vacuo* to approximately 20 mL, and *i*Pr_2EtN (1 mL) was added to quench residual acid. The mixture was then concentrated to dryness, affording crude **SI-4**, which is commercially available (e.g. from Sigma-Aldrich).

Crude **SI-4** was dissolved in 100 mL of MeCN, and *i*Pr_2EtN (8.4 g, 11.3 mL, 65.1 mmol, 1.2 equiv.), Bu_2SnCl_2 (836 mg, 2.8 mmol, 0.1 equiv.), and *p*-TsCl (11.5 g, 60.2 mmol, 1.1 equiv.) were added sequentially. The reaction mixture was stirred at room temp. for 2 hr, quenched with the addition of aq. sat. $NaHCO_3$ (100 mL), and extracted with EtOAc (100 mL x 3). The organic layers were filtered through a 1.5" pad of silica and concentrated *in vacuo*. The resulting colorless oil was dissolved in THF (100 mL), and NaH (4.4 g, 110 mmol, 2.0 equiv.) was added in approximately 10 small portions to avoid extreme exothermic conditions during H_2 gas release. The resulting yellow suspension was stirred for 15 min., and $LiAlH_4$ (2.4 g, 63.2 mmol, 1.1 equiv.) was added in approximately 10 portions, careful to avoid extremely quick gas or heat release. An additional 100 mL of THF was added to facilitate stirring as the suspension turned into a thick foam. The reaction mixture was then refluxed for 1 hr using a mineral oil bath, cooled to room temp., and sat. aq. Na_2SO_4 was added dropwise until bubbling ceased. AcOH was added until the pH of the crude reaction mixture was roughly 7, and the mixture was then filtered through a 1.5" pad of silica. The obtained products were a mixture of a yellow liquid (target product) and a separate phase of clear oil (from the NaH), which was removed by washing with hexanes. The remaining yellow oil was dissolved in 100 mL of pyridine, and BzCl (15 mL, 129 mmol, 2.3 equiv.) was added dropwise at 0 °C. The reaction mixture was stirred at room temp. for 24 hr, then sat. aq. $NaHCO_3$ (200 mL) was added, and the reaction was stirred for 2 hr, evaporated *in vacuo*, and extracted with hot hexanes. The hexane extracts were dried over Na_2SO_4, filtered, and evaporated to dryness. Flash column chromatography on silica using 10% EtOAc in hexanes was performed affording **15** (9.1 g, 24.7 mmol, 45%) as a colorless oil.

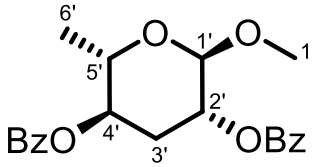
Use of moisture-free techniques with fresh $LiAlH_4$ and NaH avoided the formation of ring contraction product **SI-5**.[14]

HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{21}H_{22}O_6Na$ 393.1309; found 393.1303.

HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{21}H_{22}O_6Na$ 393.1309; found 393.1300.

See Tables S4-5 for NMR spectroscopic assignments of **15** and **SI-5**.

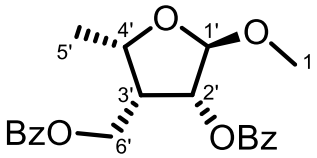
Table S4. NMR spectroscopic data for 2,4-di-*O*-benzoyl-1-*O*-methyl ascarylose (15). ^1H (800 MHz), HSQC, and HMBC NMR spectroscopic data were acquired in methanol-d_4. Chemical shifts were referenced to δ(C**H**D_2OD) = 3.31 ppm and δ(^{13}**C**HD_2OD) = 49.00 ppm. ^1H NMR data for aromatic protons: δ 8.10 (m, 2H), 8.02 (m, 2H), 7.65 (m, 1H), 7.62 (m, 1H), 7.53 (m, 2H), 7.49 (m, 2H). ^{13}C NMR chemical shift data for aromatic and ester carbons: δ 166.8, 166.7, 134.4, 134.3, 130.9, 130.4, 130.3, 129.4.



15

Position	^{13}C [ppm]	^1H [ppm]	$J_{H,H}$ couplings [Hz]	HMBC correlations
1	55.1	3.48		C-1'
1'	98.5	4.73	$J_{1',2'}$ = 2.6	C-1, C-2', C-3', C-5'
2'	71.6	5.15	$J_{2',3'a}$ = 3.0 $J_{2',3'b}$ = 3.4	C-1', C-3', C-4'
3'	30.3	2.20 (a), 2.40 (b)	$J_{3'a,3'b}$ = 13.1 $J_{3'a,4'}$ = 11.5 $J_{3'b,4'}$ = 3.6	C-1', C-2', C-4', C-5'
4'	71.6	5.12	$J_{4',5'}$ = 9.6	C-3', C-5', C-6'
5'	67.6	4.08	$J_{5',6'}$ = 6.6	C-1', C-3', C-4', C-6'
6'	17.9	1.28		C-4', C-5'

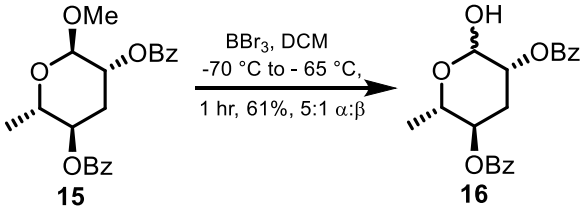
Table S5. NMR spectroscopic data for SI-5. ^{1}H (600 MHz) and HMBC NMR spectroscopic data were acquired in chloroform-*d* and NOESY (800 MHz) in methanol-d_4. Chemical shifts were referenced to δ(C**H**Cl_3) = 7.26, δ(^{13}**C**HCl_3) = 77.2, and δ(C**H**D_2OD) = 3.31 ppm. ^{1}H NMR data for aromatic protons (chloroform-*d*): δ 8.02 – 7.96 (m, 4H), 7.59 – 7.53 (m, 2H), 7.46 – 7.39 (m, 4H). ^{1}H NMR data for aromatic protons (methanol-d_4): δ 7.98 – 7.95 (m, 2H), 7.94 – 7.91 (m, 2H), 7.47 – 7.43 (m, 4H), 7.43 – 7.40 (m, 2H). ^{13}C NMR chemical shift data for aromatic carbons (chloroform-*d*): δ 161.5, 160.7, 128.6, 128.4, 124.8, 124.4, 123.7, 123.4.



SI-5

Position	^{13}C [ppm]	^{1}H [ppm] ($CDCl_3$)	$J_{H,H}$ couplings [Hz]	HMBC correlations	^{1}H [ppm] (CD_3OD)	$J_{H,H}$ couplings [Hz]	NOESY correlations
1	50.1	3.42	-	C-1'	3.39	-	H-1'
1'	101.3	5.06	-	C-1, C-3', C-4'	5.02	-	H-2'
2'	73.0	5.51	$J_{2',3'}$ = 5.2	C-4', C-6'	5.47	$J_{2',3'}$ = 5.1	H-3'
3'	36.8	3.22	$J_{3',4'}$ = 7.7, $J_{3',6'}$ = 7.5	C-4', C-5', C-6'	3.18	$J_{3',4'}$ = 7.8, $J_{3',6'a}$ = 6.8, $J_{3',6'b}$ = 8.5	H-4', $H_{a,b}$-6'
4'	70.8	4.62	$J_{4',5'}$ = 6.6	C-2'	4.58	$J_{4',5'}$ = 6.7	H-5'
5'	13.4	1.43	-	C-3', C-4'	1.39	-	$H_{a,b}$-6'
6'	55.2	4.54	-	C-2', C-3', C-4'	4.50 (a), 4.54 (b)	$J_{6'a,6'b}$ = 11.2	-

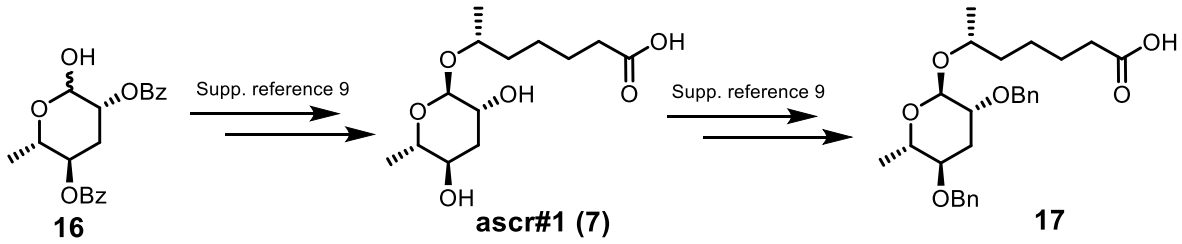
2,4-Di-*O*-benzoyl ascarylose (16)



Under Ar, BBr_3 (1M in DCM, 4 mL, 4.0 mmol, 3.7 equiv.) was added dropwise over a 15 min. period to a solution of **15** (400 mg, 1.08 mmol, 1.0 equiv.) in DCM, previously cooled to -70 °C. The reaction mixture was stirred at -65 °C for 1 hr and quenched by transferring to cold sat. aq. $NaHCO_3$ (5 mL). The organics were extracted with DCM (5 mL x 3), dried with $MgSO_4$, filtered through celite, and concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 0-50% EtOAc in hexanes afforded **16** (235 mg, 5:1 α:β, 61%) as a colorless, viscous oil. NMR spectroscopic data were consistent to those previously reported.[15]

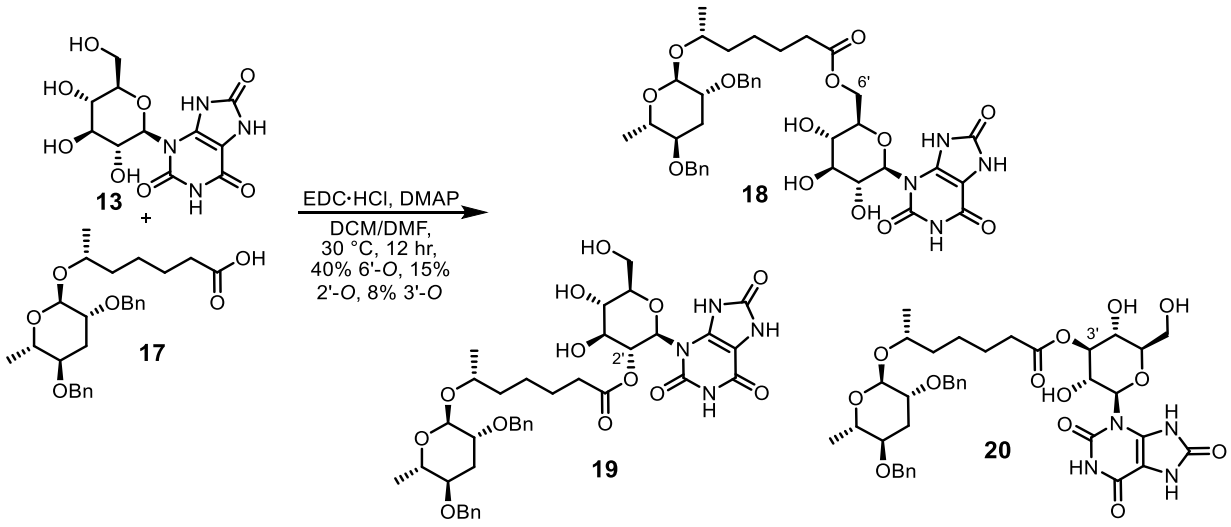
3.3. Non-selective synthesis of uglas isomers 21-23

2′,4′-Di-*O*-benzyl ascr#1 (17)



Dibenzylated ascr#1 (**17**) was prepared starting from **16** according to a previously published procedure, with NMR spectra consistent with previously reported data.[16]

2‴-4‴-Di-*O*-benzyl uglas#1 isomers (18-20)



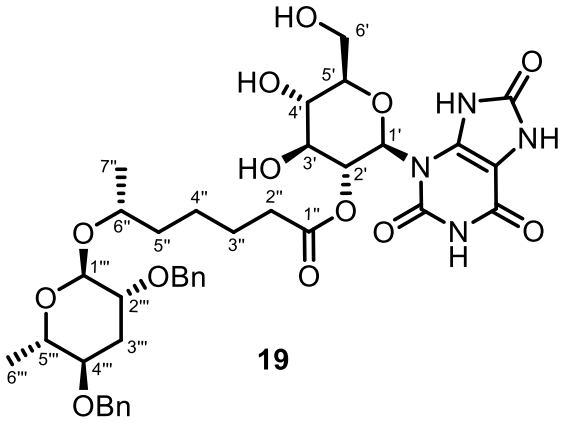
A solution of gluric#1 (**13**, 6.5 mg, 0.020 mmol, 2.0 equiv.) in 350 µL DMF and 350 µL DCM were added to **17** (4.5 mg, 0.010 mmol, 1.0 equiv.) in 130 µL DCM and 4-dimethylaminopyridine (5.0 mg, 0.041 mmol, 4.1 equiv.) in 100 µL DCM. The solution was stirred for 5 min., then EDC•HCl (5.5 mg, 0.029 mmol, 2.9 equiv.) in 300 µL DMF and 200 µL DCM were added, and the resulting solution was stirred at 30 °C for 12 hr using a mineral oil bath. The solution was concentrated *in vacuo* followed by flash column chromatography on silica using a gradient of 0-60% MeOH in DCM (with 0.2% AcOH), affording several fractions containing 2'-*O* (**19**, 1.2 mg, 15%), 3'-*O* (**20**, 0.6 mg, 8%) and 6'-*O* (**18**, 3.0 mg, 40%) isomers, with good separation of 2'-*O* and 3'-*O* isomers from the 6'-*O* isomer.

^{1}H NMR (6'-*O* isomer, 18) (600 MHz, methanol-d_4): δ 7.37 – 7.24 (m, 10H), 5.62 (br s, 1H), 4.71 (br s, 1H), 4.56 (d, *J* = 11.7 Hz, 1H), 4.52 (s, 2H), 4.46 (dd, *J* = 12.1, 2.2 Hz, 1H), 4.45 (d, *J* = 11.5), 4.27 (dd, *J* = 12.1, 7.2 Hz, 1H), 3.87 (br s, 1H), 3.73 – 3.68 (m, 2H), 3.68 – 3.62 (m, 1H), 3.57 (t, *J* = 9.4 Hz, 1H), 3.50 (m, 1H), 3.37 (ddd, *J* = 10.8, 9.5, 4.1 Hz, 1H), 2.35 (dt, *J* = 7.5, 3.6 Hz, 2H), 2.23 (dt, *J* = 13.4, 4.0 Hz, 1H), 1.63 (ddd, *J* = 13.4, 10.9, 2.9 Hz, 1H), 1.61 – 1.30 (m, 6H), 1.18 (d, *J* = 6.2 Hz, 3H), 1.04 (d, *J* = 6.1 Hz, 3H). ^{1}H NMR spectroscopic data of **18** are consistent with data previously reported for the related iglas#1.[17]

HRMS (ESI) *m/z*: $[M - H]^-$ calcd for $C_{38}H_{47}O_{13}N_4$ 767.3145; found 767.3135 for all isomers.

See Tables S6 and S7 for NMR spectroscopic assignments of **19** and **20**.

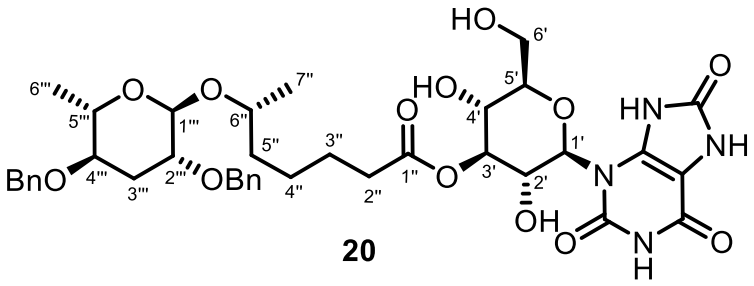
Table S6. NMR spectroscopic data for 2‴-4‴-di-*O*-benzyl uglas#1 (19). ^{1}H (600 MHz) data were acquired in methanol-d_4. Chemical shifts were referenced to δ(C**H**D_2OD) = 3.31 ppm. ^{1}H NMR data for aromatic and benzylic protons: δ 7.38 – 7.32 (m, 8H), 7.32 – 7.27 (m, 2H), 4.58 (d, J = 11.7 Hz, 1H), 4.53 (s, 2H), 4.48 (d, J = 11.7 Hz, 1H). Note: extreme line broadening prevented assignment of some protons (marked #).



19

Position	^{1}H [ppm]	$J_{H,H}$ couplings [Hz]
1'	5.88	$J_{1',2'}$ (weak)
2'	5.20	$J_{2',3'}$ (weak)
3'	3.70	-
4'	#	-
5'	#	-
6'	#	-
1"	-	-
2"	2.27	$J_{2",3"}$ = 7.4
3"	1.53	-
4"	1.30	-
5"	-	-
6"	3.75	$J_{6",7"}$ = 6.1
7"	1.06	-
1‴	4.75	$J_{1‴,2‴}$ = 2.5
2‴	3.53	$J_{2‴,3‴a}$ = 3.5, $J_{2‴,3‴b}$ = 3.0
3‴	1.65 (a), 2.24 (b)	$J_{3‴a,3‴b}$ = 13.6, $J_{3‴a,4‴}$ = 10.8, $J_{3‴b,4‴}$ = 4.3
4‴	3.39	$J_{4‴,5‴}$ = 9.0
5‴	3.73	$J_{5‴,6‴}$ = 6.3
6‴	1.21	-

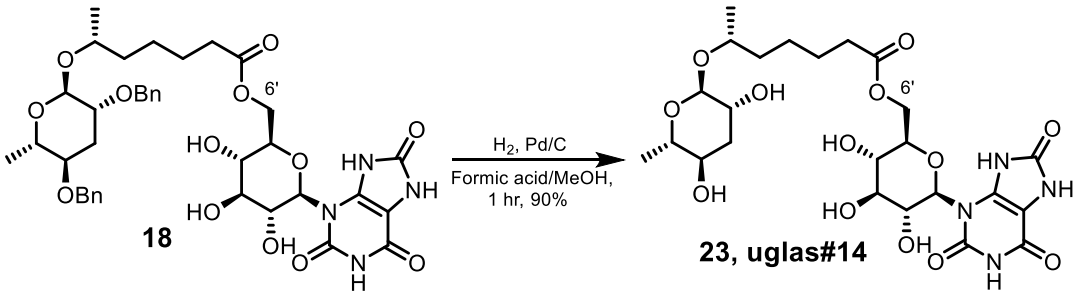
Table S7. NMR spectroscopic data for 2‴-4‴-di-*O*-benzyl uglas#12 (20). ^{1}H (600 MHz) data were acquired in methanol-d_4. Chemical shifts were referenced to δ(C**H**D$_2$OD) = 3.31 ppm. ^{1}H NMR data for aromatic and benzylic protons: δ 7.38 – 7.32 (m, 8H), 7.32 – 7.27 (m, 2H), 4.56 (d, *J* = 11.7 Hz, 1H), 4.53 (s, 2H), 4.46 (d, *J* = 11.7 Hz, 1H). Note: extreme line broadening prevented assignment of some protons (marked #).



20

Position	^{1}H [ppm]	$J_{H,H}$ couplings [Hz]
1'	5.75	$J_{1',2'}$ = 9.5
2'	4.15	$J_{2',3'}$ = 9.5
3'	5.06	$J_{3',4'}$ = 9.4
4'	3.80	-
5'	3.56	-
6'	#	-
1"	-	-
2"	2.42	$J_{2",3"}$ = 7.4
3"	1.66	-
4"	-	-
5"	-	-
6"	3.75	$J_{6",7"}$ = 6.1
7"	1.07	-
1‴	4.73	$J_{1‴,2‴}$ = 2.4
2‴	3.53	$J_{2‴,3‴a}$ = 3.5, $J_{2‴,3‴b}$ = 3.0
3‴	1.65 (a), 2.24 (b)	$J_{3‴a,3‴b}$ = 13.6, $J_{3‴a,4‴}$ = 10.8, $J_{3‴b,4‴}$ = 4.3
4‴	3.39	$J_{4‴,5‴}$ = 9.0
5‴	3.73	$J_{5‴,6‴}$ = 6.2
6‴	1.21	-

uglas#14 (23)

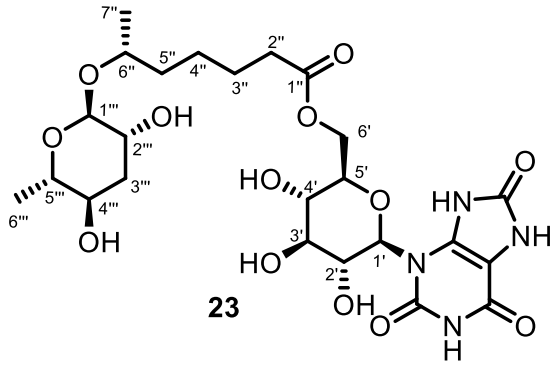


To a solution of **18** (3.0 mg, 0.004 mmol) in 1.8 mL MeOH was added Pd/C (10% w/w) (4.0 mg) and formic acid (two drops). The reaction mixture was purged with Ar for 5 min., subjected to H_2 for 1 hr at room temp., and again purged with Ar for 5 min. The reaction mixture was filtered through celite and the filtrate was concentrated *in vacuo* affording crude uglas#14 (**23**, 2.0 mg, 90%), as a colorless oil. The crude reaction mixture of **23** (uglas#14) was analyzed using HPLC-HRMS on a C18 column and retention times of isomers of uglas#1 in the synthetic mixture were compared to those of isomers in *C. elegans* wildtype (N2) *endo*-metabolome samples (see Figure 3b).

See Table S8 for NMR spectroscopic assignments of **23**.

HRMS (ESI) *m/z*: $[M - H]^-$ calcd for $C_{24}H_{35}O_{13}N_4$ 587.2206; found 587.2218.

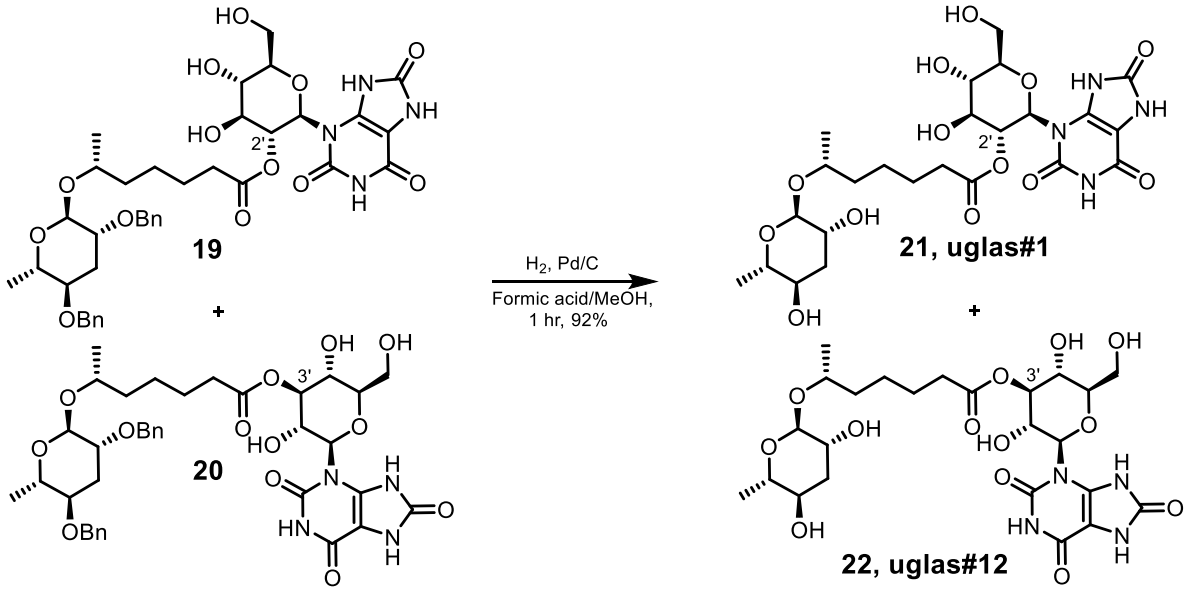
Table S8. NMR spectroscopic data for uglas#14 (23). ^{1}H (800 MHz), ^{13}C (201 MHz), HSQC, and HMBC NMR spectroscopic data were acquired in methanol-d_4. Chemical shifts were referenced to δ(C**H**D_2OD) = 3.31 ppm and δ(^{13}**C**HD_2OD) = 49.00 ppm. ^{13}C NMR chemical shift data for uric acid carbons: δ 154.6, 154.1, 150.7, 134.8, 100.7.



23

Position	^{13}C [ppm]	^{1}H [ppm]	$J_{H,H}$ couplings [Hz]	HMBC correlations
1'	85.1	5.62 (broad)	-	-
2'	70.9	3.86	$J_{2',3'}$ = 9.2	-
3'	78.3	3.49	$J_{3',4'}$ = 9.5	C-1', C-2', C-4', C-5',
4'	70.8	3.58	$J_{4',5'}$ = 10.5	C-3', C-5', C-6', C-1"
5'	79.0	3.71	$J_{5',6'a}$ = 7.5, $J_{5',6'b}$ = 2.0	C-3'
6'	64.7	4.30 (a), 4.48 (b)	$J_{6'a,6'b}$ = 12.1	C-4' (a,b), C-5''' (a)
1"	175.0	-	-	-
2"	34.5	2.37	$J_{2",3"}$ = 7.4	C-1", C-3"
3"	25.7	1.61	-	C-1", C-2", C-4", C-5"
4"	25.9	1.37, 1.43	-	C-2", C-3", C-5", C-6",
5"	37.6	1.43 (a), 1.53 (b)	$J_{5"a,6"}$ = 7.4, $J_{5"b,6"}$ = 4.7	C-3", C-4", C-6", C-7"
6"	72.0	3.72	$J_{6",7"}$ = 6.1	C-4", C-5", C-1'''
7"	19.0	1.09	-	C-5", C-6"
1'''	97.3	4.61	$J_{1''',2'''}$ = 2.6	C-6", C-2''', C-3''', C-5'''
2'''	69.7	3.70	$J_{2''',3'''a}$ = 3.4, $J_{2''',3'''b}$ = 3.2	C-1''', C-3'''
3'''	35.7	1.75 (a), 1.93 (b)	$J_{3'''a,3'''b}$ = 13.1, $J_{3'''a,4'''}$ = 11.0, $J_{3'''b,4'''}$ = 3.7	C-1''' (b), C-2''' (b), C-4''' (a,b), C-5''' (a,b)
4'''	68.1	3.49	$J_{4''',5'''}$ = 9.6	C-3''', C-5''', C-6'''
5'''	71.0	3.58	$J_{5''',6'''}$ = 6.2	C-1''', C-3''', C-4''', C-6'''
6'''	17.8	1.19	-	C-4''', C-5'''

uglas#1 (21) and uglas#12 (22)

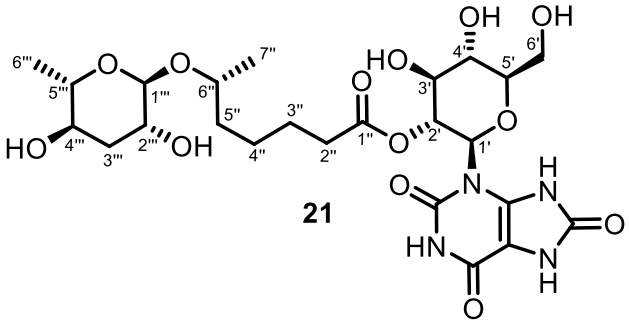


To a solution containing a 2:1 mixture of **19** and **20** (1.8 mg, 0.0024 mmol), respectively, in 0.9 mL MeOH was added Pd/C (10% w/w) (2.0 mg) and formic acid (one drop). The reaction mixture was purged with Ar for 5 min, subjected to H_2 for 1 hr at room temp., and then purged with Ar for 5 min. The reaction mixture was filtered through celite, and the filtrate was concentrated *in vacuo*., affording a 2:1 mixture of **21** and **22** (1.2 mg, 92%), respectively, as a colorless oil. The residue was analyzed using HPLC-HRMS on a C18 column and retention times of uglas#1/12 (**21** and **22**) isomers were compared to those of isomers in *C. elegans* wildtype (N2) *endo*-metabolome samples (see Figure 3b).

See Table S9 for NMR spectroscopic assignments of **21**.

HRMS (ESI) *m/z*: $[M - H]^-$ calcd for $C_{24}H_{35}O_{13}N_4$ 587.2206; found 587.2218 for both isomers.

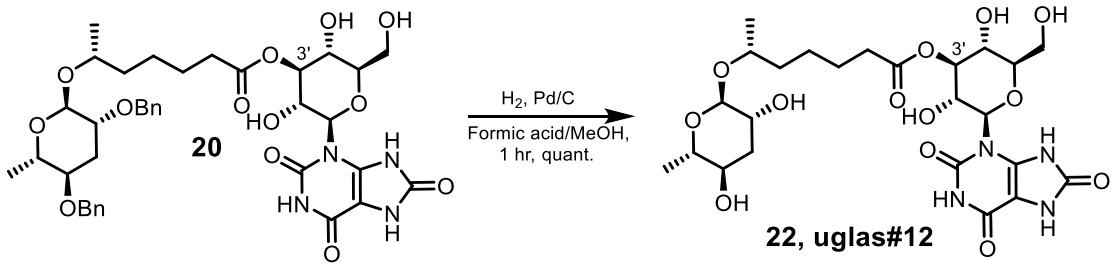
Table S9. NMR spectroscopic data for uglas#1 (21). ^{1}H (800 MHz), ^{13}C (201 MHz), HSQC, and HMBC NMR spectroscopic data were acquired in methanol-d_4. Chemical shifts were referenced to δ(C**H**D_2OD) = 3.31 ppm and δ(^{13}**C**HD_2OD) = 49.00 ppm. ^{13}C NMR chemical shift data for uric acid carbons: δ 155.0, 154.1, 150.6, 100.7. One of the uric acid carbons expected at ~134 ppm could not be observed due to line broadening.



21

Position	^{13}C [ppm]	^{1}H [ppm]	$J_{H,H}$ couplings [Hz]	HMBC correlations
1'	82.5	5.91	-	-
2'	72.2	5.20	-	-
3'	76.1	3.71	-	C-4'
4'	69.9	3.77	-	-
5'	81.7	3.56	$J_{5',6'}$ = 2.5	-
6'	61.5	3.89, 3.90	-	C-4', C-5'
1"	174.1	-	-	-
2"	34.5	2.29	$J_{2",3"}$ = 9.1	C-1", C-3"
3"	25.6	1.52	-	-
4"	25.9	-	-	-
5"	37.6	1.43, 1.52	$J_{5",6"}$ = 6.8	C-3", C-4", C-6", C-7"
6"	71.9	3.75	$J_{6",7"}$ = 6.1	C-5", C-1'"
7"	19.1	1.10	-	C-5", C-6"
1'"	97.3	4.65	$J_{1'",2'"}$ = 2.7	C-6", C-2'", C-3'", C-5'"
2'"	69.7	3.71	$J_{2'",3'"a}$ = 3.0, $J_{2'",3'"b}$ = 3.1	C-1'", C-3", C-4'"
3'"	35.6	1.76 (a), 1.94 (b)	$J_{3'"a,3'"b}$ = 13.1, $J_{3'"a,4'"}$ = 11.3, $J_{3'"b,4'"}$ = 4.6	C-1'" (b), C-2'" (a,b), C-4'" (a,b), C-5'" (a,b)
4'"	68.1	3.51	$J_{4'",5'"}$ = 9.6	C-3'", C-5'", C-6'"
5'"	70.9	3.60	$J_{5'",6'"}$ = 6.2	C-1'", C-3'", C-4'", C-6'"
6'"	17.8	1.22	-	C-4'", C-5'"

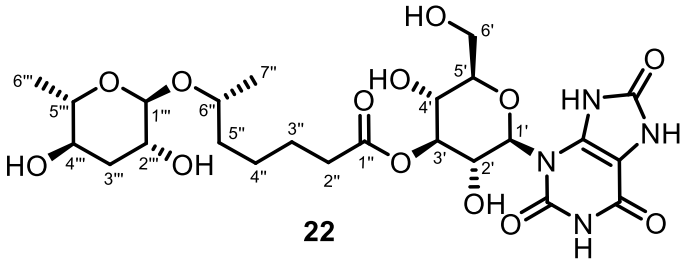
uglas#12 (22)



To a solution of containing **20** (1.6 mg, 0.0024 mmol), respectively, in 1.0 mL MeOH was added Pd/C (10% w/w) (2.0 mg) and formic acid (two drops). The reaction mixture was purged with Ar for 5 min, subjected to H_2 for 1 hr at room temp., and then purged with Ar for 5 min. The reaction mixture was filtered through celite, and the filtrate was concentrated *in vacuo*., affording crude uglas#12 (**22,** 1.2 mg, quant.) as a colorless oil. The residue was analyzed using HPLC-HRMS on a C18 column and the retention times of uglas#12 (**22**) was compared to those of isomers in *C. elegans* wildtype (N2) *endo*-metabolome samples (see Figure S8).

See Table S10 for NMR spectroscopic assignments of **22**.

Table S10. NMR spectroscopic data for uglas#12 (22). ^{1}H (800 MHz), ^{13}C (201 MHz), HSQC, and HMBC NMR spectroscopic data were acquired in methanol-d_4. Chemical shifts were referenced to δ(C**H**D_2OD) = 3.31 ppm and δ(^{13}**C**HD_2OD) = 49.00 ppm. ^{13}C NMR chemical shift data for uric acid carbons: δ 154.5, 150.8, 100.8. Two additional uric acid carbons could not be observed due to line broadening.

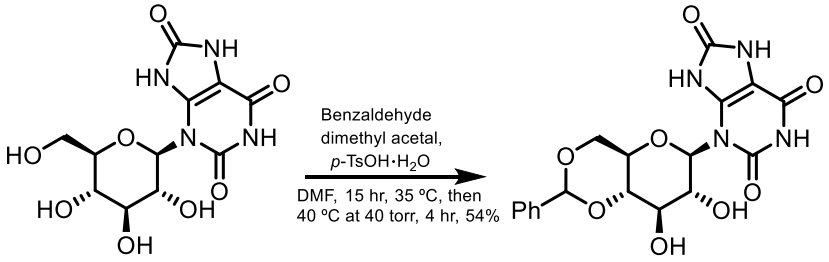


22

Position	^{13}C [ppm]	^{1}H [ppm]	$J_{H,H}$ couplings [Hz]	HMBC correlations
1'	85.2	5.76	$J_{1',2'}$ = 9.3	-
2'	69.3	3.99	$J_{2',3'}$ = 9.3	-
3'	79.0	5.08	$J_{3',4'}$ = 9.5	C-1', C-2', C-4', C-5', C-1"
4'	68.1	3.83	$J_{4',5'}$ = 9.4	C-3', C-5', C-6', C-1"
5'	81.4	3.60	-	C-1', C-3', C-4'
6'	61.3	3.87, 3.88	-	C-4', C-5'
1"	174.7	-	-	-
2"	34.9	2.44	$J_{2'',3''}$ = 7.4	C-1", C-3"
3"	25.7	1.69	-	C-1", C-2", C-4", C-5"
4"	26.1	-	-	-
5"	37.8	1.48, 1.58	$J_{5'',6''}$ = 7.1	C-3", C-4", C-6", C-7"
6"	72.0	3.79	$J_{6'',7''}$ = 6.1	C-4", C-5", C-1'"
7"	19.0	1.12	-	C-5", C-6"
1'"	97.2	4.64	$J_{1''',2'''}$ = 2.7	C-6", C-2'", C-3'", C-5'"
2'"	69.7	3.71	$J_{2''',3'''a}$ = 3.3, $J_{2''',3'''b}$ = 3.0	C-1'", C-3'"
3'"	35.7	1.76 (a), 1.94 (b)	$J_{3'''a,3'''b}$ = 13.1, $J_{3'''a,4'''}$ = 11.3, $J_{3'''b,4'''}$ = 3.6	C-1'" (b), C-2'" (b), C-4'" (a,b), C-5'" (a,b)
4'"	68.1	3.51	$J_{4''',5'''}$ = 9.3	C-3'", C-5'", C-6'"
5'"	70.9	3.60	$J_{5''',6'''}$ = 6.3	C-1'", C-3'", C-4'", C-6'"
6'"	17.8	1.21	-	C-4'", C-5'"

3.4. Selective synthesis of uglas#1 (21) and uglas#11 (26)

4′,6′-*O*-Benzylidene-N^3-(*β*-glucopyranosyl)uric acid (SI-6)



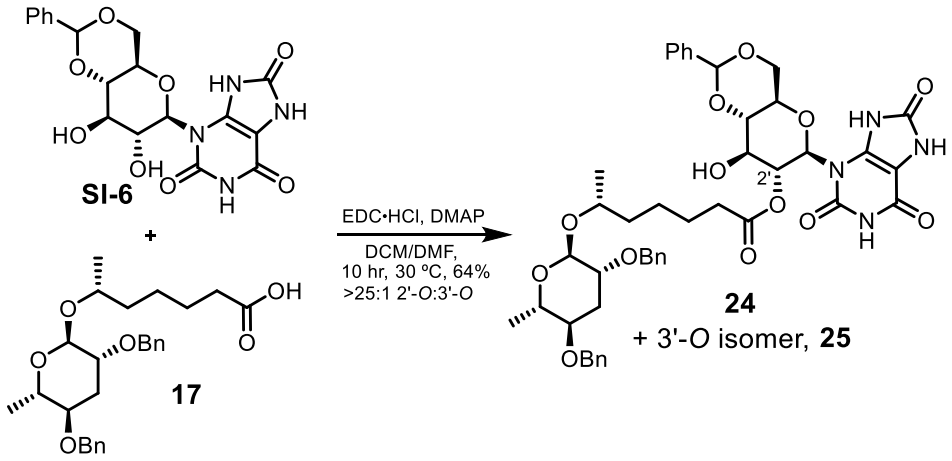
13, gluric#1 **SI-6**

To a solution of gluric#1 (**13**, 40 mg, 0.120 mmol, 1.0 equiv.) in 0.6 mL DMF was added benzaldehyde dimethyl acetal (44 µL, 0.293 mmol, 2.4 equiv.) and *p*-TsOH•H_2O (3.0 mg, 0.016 mmol, 0.1 equiv.) in 50 µL DMF. The solution was stirred for 20 hr at 35 ºC using a mineral oil bath and then an additional 40 µL of benzaldehyde dimethyl acetal (0.267 mmol, 2.2 equiv.) was added. The reaction mixture was then heated to 40 ºC and allowed to run under reduced pressure (40 torr) using a rotary evaporator for 4 hr. The reaction mixture was then concentrated completely *in vacuo*. The crude material was reconstituted in DCM/MeOH, triethylamine (10 µL) was added, and flash column chromatography on silica using a gradient of 0-40% MeOH in DCM was performed, affording **SI-6** (27 mg, 54%) as a white solid and recovered **13** (12 mg, 30%).

^{1}H NMR (600 MHz, methanol-d_4): δ 7.55 – 7.50 (m, 2H), 7.39 – 7.32 (m, 3H), 5.75 (br. s, 1H), 5.63 (s, 1H), 4.30 (dd, *J* = 10.2, 5.0 Hz, 1H), 4.10 – 3.82 (m, 3H), 3.76 (t, *J* = 8.9 Hz, 1H), 3.67 (dt, *J* = 9.5, 5.0 Hz, 1H).

HRMS (ESI) *m/z*: $[M - H]^-$ calcd for $C_{18}H_{17}O_8N_4$ 417.1052; found 417.1049.

4′,6′-*O*-Benzylidene-2‴,4‴-di-*O*-benzyl uglas#1 (24)

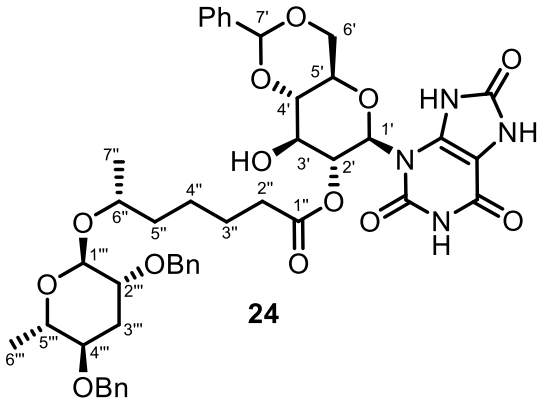


A solution of **SI-6** (11 mg, 0.026 mmol, 2.6 equiv.) in 300 µL DMF and 150 µL DCM was added to **17** (4.5 mg, 0.010 mmol, 1.0 equiv.) and 4-dimethylaminopyridine (5.5 mg, 0.045 mmol, 4.5 equiv.) in 190 µL DCM. The solution was stirred for 5 min, then EDC•HCl (5.7 mg, 0.030 mmol, 3.0 equiv.) in 200 µL DCM was added, and the resulting solution was stirred at 30 °C using a

mineral oil bath for 10 hr. The solution was concentrated *in vacuo*, and flash column chromatography on silica using a gradient of 0-30% MeOH in DCM was performed, affording **24** and trace amounts of its 3'-*O* isomer (**25**) (>25:1) (5.5 mg, 64%) as a colorless oil. See Table S11 for NMR spectroscopic assignments of **24**.

HRMS (ESI) *m/z*: $[M - H]^-$ calcd for $C_{45}H_{51}O_{13}N_4$ 855.3458; found 855.3455.

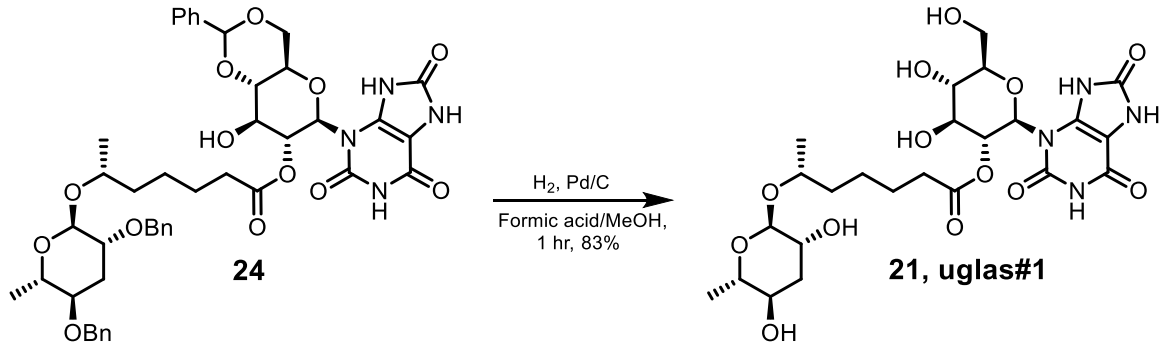
Table S11. NMR spectroscopic data for 24. ^{1}H (800 MHz), ^{13}C (201 MHz), HSQC, and HMBC data were acquired in methanol-d_4. Chemical shifts were referenced to δ(C**H**D_2OD) = 3.31 ppm and δ(**^{13}C**HD_2OD) = 49.00 ppm. ^{1}H NMR data for aromatic and benzylic protons: δ 7.54 – 7.50 (m, 2H), 7.37 – 7.30 (m, 11H), 7.30 – 7.25 (m, 2H), 4.56 (d, J = 11.7 Hz, 1H), 4.52 (s, 2H), 4.46 (d, J = 11.7 Hz, 1H). ^{13}C NMR chemical shift data for aromatic, benzylic, and uric acid carbons: δ 154.2, 150.3, 139.5, 138.6, 129.7, 129.2, 129.1, 128.8, 128.6, 127.2, 102.8, 72.0, 71.9.



24

Position	^{13}C [ppm]	^{1}H [ppm]	$J_{H,H}$ couplings [Hz]	HMBC correlations
1'	82.5	6.06	$J_{1',2'}$ = 8.5	-
2'	72.3	5.28	$J_{2',3'}$ = 9.5	-
3'	72.7	4.05	$J_{3',4'}$ = 9.5	-
4'	80.2	4.15	$J_{4',5'}$ = 9.5	-
5'	71.8	3.76	$J_{5',6'b}$ = 5.0	-
6'	68.5	3.96 (a), 4.31 (b)	$J_{6'a,6'b}$ = 10.2	C-4' (b), C-5' (b), C-7' (b)
7'	102.8	5.64		C-4', C-6'
1''	175.2			-
2''	34.4	2.30	$J_{2'',3''}$ = 7.4	-
3''	25.6	1.54	-	
4''	26.0	1.34	-	
5''	37.5	1.41, 1.51,	-	-
6''	72.7	3.75	$J_{6'',7''}$ = 6.2	
7''	19.1	1.05		C-5'', C-6''
1'''	95.5	4.75	$J_{1''',2'''}$ = 2.7	C-6'', C-3''', C-5'''
2'''	77.0	3.52	$J_{2''',3'''a}$ = 3.5, $J_{2''',3'''b}$ = 3.0	C-4'''
3'''	30.0	1.66 (a), 2.24 (b)	$J_{3'''a,3'''b}$ = 13.5, $J_{3'''a,4'''}$ = 11.0, $J_{3'''b,4'''}$ = 4.3	C-1''' (b), C-2''' (b), C-4''' (a,b), C-5''' (a,b)
4'''	76.0	3.39	$J_{4''',5'''}$ = 8.8	C-5'''
5'''	69.5	3.73	$J_{5''',6'''}$ = 6.1	C-1'''
6'''	18.1	1.21		C-4''', C-5'''

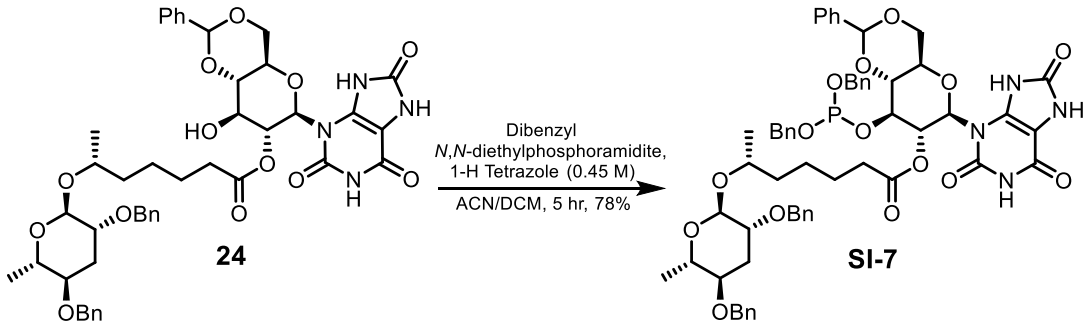
uglas#1 (21)



To a solution of **24** (3.5 mg, 0.004 mmol) in 1.7 mL MeOH was added Pd/C (10% w/w) (4.9 mg) and formic acid (four drops). The reaction mixture was purged with Ar for 5 min., subjected to H_2 for 1 hr at room temp., then purged with Ar for 5 min. The reaction mixture was filtered through celite, the filtrate was concentrated *in vacuo*, and flash column chromatography on silica using a gradient of 0-80% MeOH in DCM was performed, affording uglas#1 (**21**, 2.0 mg, 83%) as a colorless oil. uglas#1 was compared to the corresponding peak in *C. elegans* wildtype (N2) *endo*-metabolome samples by HPLC-HRMS and MS^2 (see Figures S7 and S8).

HRMS (ESI) *m/z*: $[M - H]^-$ calcd for $C_{24}H_{35}O_{13}N_4$ 587.2206; found 587.2218.

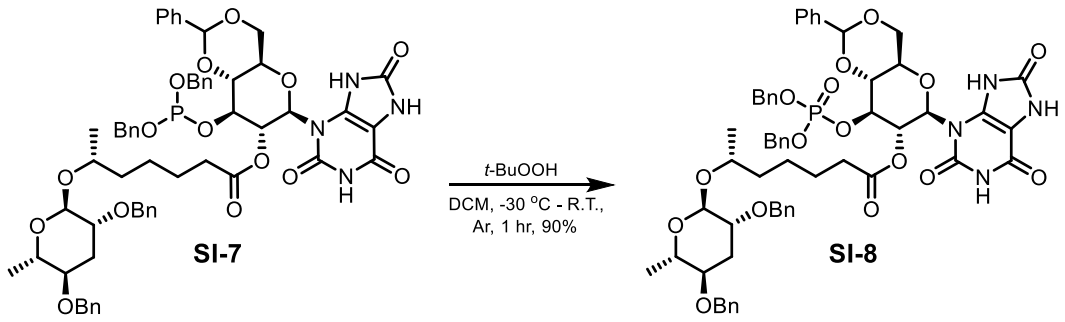
4′,6′-*O*-Benzylidene-3′-(di-*O*-benzylphosphityl)-2‴,4‴-di-*O*-benzyl uglas#1 (SI-7)



To a solution of **24** (3 mg, 0.0035 mmol, 1.0 equiv.) in 0.3 mL ACN and 0.1 mL DCM was added dibenzyl *N*,*N*-diethylphosphoramidite (3.2 µL, 0.011 mmol, 3.1 equiv.) and 1H-tetrazole (0.45 M in ACN, 46.5 µL, 0.021 mmol, 6.0 equiv.). The solution was stirred at room temp. for 5 hr and then concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 0-30% MeOH in DCM afforded **SI-7** (3.0 mg, 78%) as a white solid.

^{1}H NMR (600 MHz, mixture of methanol-*d*$_4$:chloroform-*d* (10:1)): δ 7.46 (m, 2H), 7.37 – 7.17 (m, 21H), 7.07 (m, 2H), 6.12 (d, *J* = 10.2 Hz, 1H), 5.62 (s, 1H), 5.45 (br s, 1H), 4.88 (dd, *J* = 12.2, 7.0 Hz, 1H), 4.76 – 4.67 (m, 4H), 4.56 (d, *J* = 11.7 Hz, 1H), 4.51 (s, 2H), 4.46 (d, *J* = 11.7 Hz, 1H), 4.36 (t, *J* = 9.0 Hz, 1H), 4.34 (dd, *J* = 10.2, 6.0 Hz, 1H), 3.96 (t, *J* = 10.1 Hz, 1H), 3.86 (m, 1H), 3.71 (m, 1H), 3.65 (m, 1H), 3.51 (m, 1H), 3.39 (ddd, *J* = 10.8, 9.5, 4.2 Hz, 1H), 2.22 (dt, *J* = 13.5, 3.5 Hz, 1H), 2.12 – 2.06 (m, 1H), 2.03 – 1.97 (m, 1H), 1.67 (ddd, *J* = 13.5, 10.9, 2.9 Hz, 1H), 1.42 – 1.27 (m, 6H), 1.22 (d, *J* = 6.1 Hz, 3H), 1.00 (d, *J* = 6.0 Hz, 3H).

4′,6′-*O*-Benzylidene-3′-(di-*O*-benzylphosphoryl)-2‴,4‴-di-*O*-benzyl uglas#1 (SI-8)

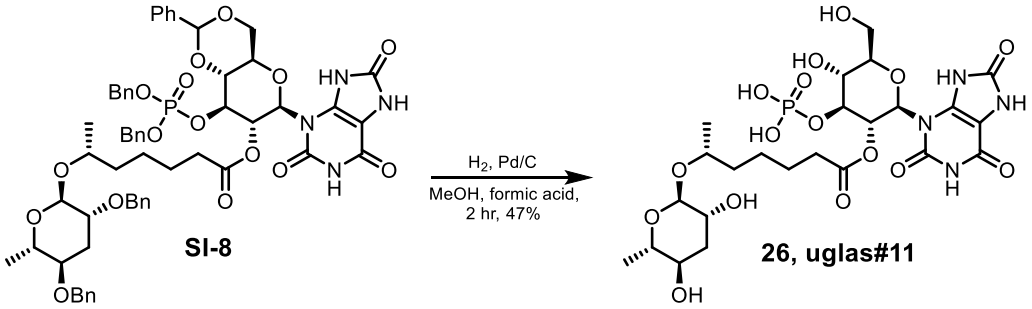


Under Ar, *tert*-butyl hydroperoxide (6M in decane) (2.5 µL, 0.015 mmol, 5.0 equiv.) was added to a solution of **SI-7** (3 mg, 0.003 mmol, 1.0 equiv.) in 0.1 mL DCM at -30 °C. The solution was stirred to up room temperature over a 1 hr period and was then concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 0-30% MeOH in DCM afforded **SI-8** (2.7 mg, 90%) as a white solid.

^{1}H NMR (600 MHz, mixture of methanol-d_4:chloroform-*d* (10:1)): δ 7.47 (m, 2H), 7.38 – 7.22 (m, 19H), 7.19 (m, 2H), 7.08 (m, 2H), 6.17 (d, *J* = 9.8 Hz, 1H), 5.64 (s, 1H), 5.51 (br s, 1H), 4.96 (m, 1H), 4.92 – 4.87 (m, 4H), 4.73 (br s, 1H), 4.56 (d, *J* = 11.7 Hz, 1H), 4.52 (s, 2H), 4.46 (d, *J* = 11.6 Hz, 1H), 4.45 (m, 1H), 4.36 (dd, *J* = 10.3, 4.9 Hz, 1H), 3.96 (m, 1H), 3.90 – 3.85 (m, 1H), 3.74 – 3.62 (m, 2H), 3.52 (m, 1H), 3.39 (ddd, *J* = 10.9, 9.5, 4.3 Hz, 1H), 2.23 (dt, *J* = 13.3, 3.9 Hz, 1H), 2.12 – 2.04 (m, 1H), 2.03 – 1.93 (m, 1H), 1.67 (ddd, *J* = 13.3, 10.9, 2.7 Hz, 1H), 1.43 – 1.27 (m, 6H), 1.22 (d, *J* = 6.2 Hz, 3H), 1.01 (d, *J* = 6.1 Hz, 3H).

HRMS (ESI) *m/z*: $[M - H]^-$ calcd for $C_{59}H_{64}O_{16}N_4P$ 1115.4060; found 1115.4099.

uglas#11 (26)

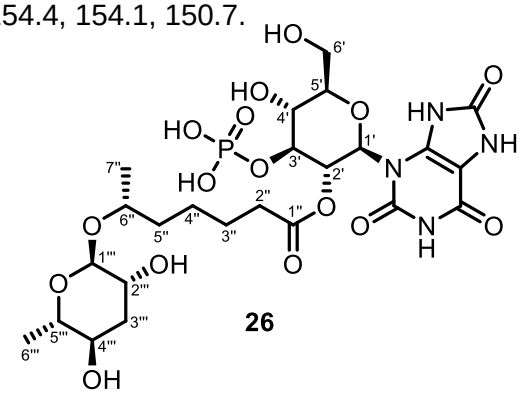


To a solution of **SI-8** (2.7 mg, 0.002 mmol) in 0.7 mL MeOH was added Pd/C (10% w/w) (3 mg) and formic acid (two drops). The reaction mixture purged with Ar for 5 min., subjected to H_2 for two hr at room temp., then purged with Ar for 5 min. After filtration through celite, the filtrate was concentrated *in vacuo*. The crude mixture was purified using preparative HPLC (see Methods), affording uglas#11 (**26**, 0.7 mg, 47%). A sample of uglas#11 was compared to the corresponding peak in *C. elegans* wildtype (N2) *endo*-metabolome samples by HPLC-HRMS and MS^2 (see Figure S10 and S11). Additionally, pure uglas#11 was used to generate standard curve (Figure S12) for concentration determination in *C. elegans endo*-metabolome samples.

See Table S12 for NMR spectroscopic assignments of **26**.

HRMS (ESI) *m/z*: $[M - H]^-$ calcd for $C_{24}H_{36}O_{16}N_4P$ 667.1869; found 667.1889.

Table 12. NMR spectroscopic data for uglas#11 (26). ^{1}H (800 MHz), ^{13}C (201 MHz), HSQC, and HMBC NMR spectroscopic data were acquired in methanol-d_4. Chemical shifts were referenced to δ(C**H**D_2OD) = 3.31 ppm and δ(^{13}**C**HD_2OD) = 49.00 ppm. ^{13}C NMR chemical shift data for uric acid carbons: δ 154.4, 154.1, 150.7.



26

Position	^{13}C [ppm]	^{1}H [ppm]	$J_{H,H}$ couplings [Hz]	HMBC correlations
1'	82.4	5.96	$J_{1',2'}$ (weak)	-
2'	70.4	5.27	$J_{2',3'}$ = 8.7	-
3'	79.5	4.37	$J_{3',4'}$ = 8.7, $J_{3',31P}$ = 8.7	C-2', C-4'
4'	70.1	3.96	$J_{4',5'}$ = 9.5	-
5'	81.8	3.60	-	-
6'	61.6	3.85, 3.89	-	C-4', C-5'
1''	174.1	-	-	-
2''	34.5	2.30, 2.36	$J_{2'',3''}$ = 7.4	C-1'', C-3''
3''	25.5	1.55	-	-
4''	26.0	1.32, 1.35	-	-
5''	37.6	1.43, 1.53		C-3'', C-4'', C-6'', C-7''
6''	72.3	3.75	$J_{6'',7''}$ = 6.1	C-3'', C-5'', C-1'''
7''	19.0	1.10	-	C-5'', C-6''
1'''	97.3	4.65	$J_{1''',2'''}$ = 2.7	C-6'', C-2''', C-3''', C-5'''
2'''	69.7	3.70	$J_{2''',3'''a}$ = 3.3, $J_{2''',3'''b}$ = 3.1	C-1''', C-3'''
3'''	35.7	1.77 (a), 1.94 (b)	$J_{3'''a,3'''b}$ = 13.1, $J_{3'''a,4'''}$ = 11.1, $J_{3'''b,4'''}$ = 3.7	C-1''' (b), C-2''' (a,b), C-4''' (a,b), C-5''' (a,b)
4'''	68.2	3.50	$J_{4''',5'''}$ = 9.4	C-3''', C-6'''
5'''	71.0	3.61	$J_{5''',6'''}$ = 6.2	C-1''', C-3''', C-4''', C-6'''
6'''	17.8	1.22	-	C-4''', C-5'''

4. Supporting references

(1) Jung, J.; Nakajima, M.; Kojima, M.; Ooe, K.; Fukuda, T. *J. Micro-Nano Mechatronics* **2012**, *7*, 3–11.

(2) Nannenga, B. L.; Shi, D.; Leslie, A. G. W.; Gonen, T. *Nat. Methods* **2014**, *11*, 927–930.

(3) Hattne, J; Reyes, F. E.; Nannenga, B. L.; Shi, D.; de la Cruz, M. J.; Leslie, A. G. W.; Gonen, T. M. *Acta Cryst.* **2015**, *A71*, 353–360.

(4) Kabsch, W. Xds. *Acta Cryst.* **2010**, *D66*, 125–132.

(5) Kabsch, W. **2010**, *D66*, 133–144.

(6) Sheldrick, G. M. *Acta Cryst.* **2008**, *A64*, 112–122.

(7) Sheldrick, G. M. *Acta Cryst.* **2015**, *C71*, 3–8.

(8) Hübschle, C. B.; Sheldrick, G. M.; Dittrich, B. *J. Appl. Cryst.* **2011**, *44*, 1281–1284.

(9) Salinas, A. E.; Sproviero, J. F.; Deulofeu, V. *Carbohydr. Res.* **1987**, *170*, 71–99.

(10) Kahn, K.; Serfozo, P.; Tipton, P. A. *J. Am. Chem. Soc.* **1997**, *119*, 5435–5442.

(11) Bonjouklian, R.; Smitka, T. A.; Doolin, L. E.; Molloy, R. M.; Debono, M.; Shaffer, S. A.; Moore, R. E.; Stewart, J. B.; Patterson, G. M. L. Tjipanazoles. *Tetrahedron* **1991**, *47*, 7739–7750.

(12) Muramatsu, W.; Tanigawa, S.; Takemoto, Y.; Yoshimatsu, H.; Onomura, O. *Chem. - A Eur. J.* **2012**, *18*, 4850–4853.

(13) Martinelli, M. J.; Vaidyanathan, R.; Van Khau, V. *Tetrahedron Lett.* **2000**, *41*, 3773–3776.

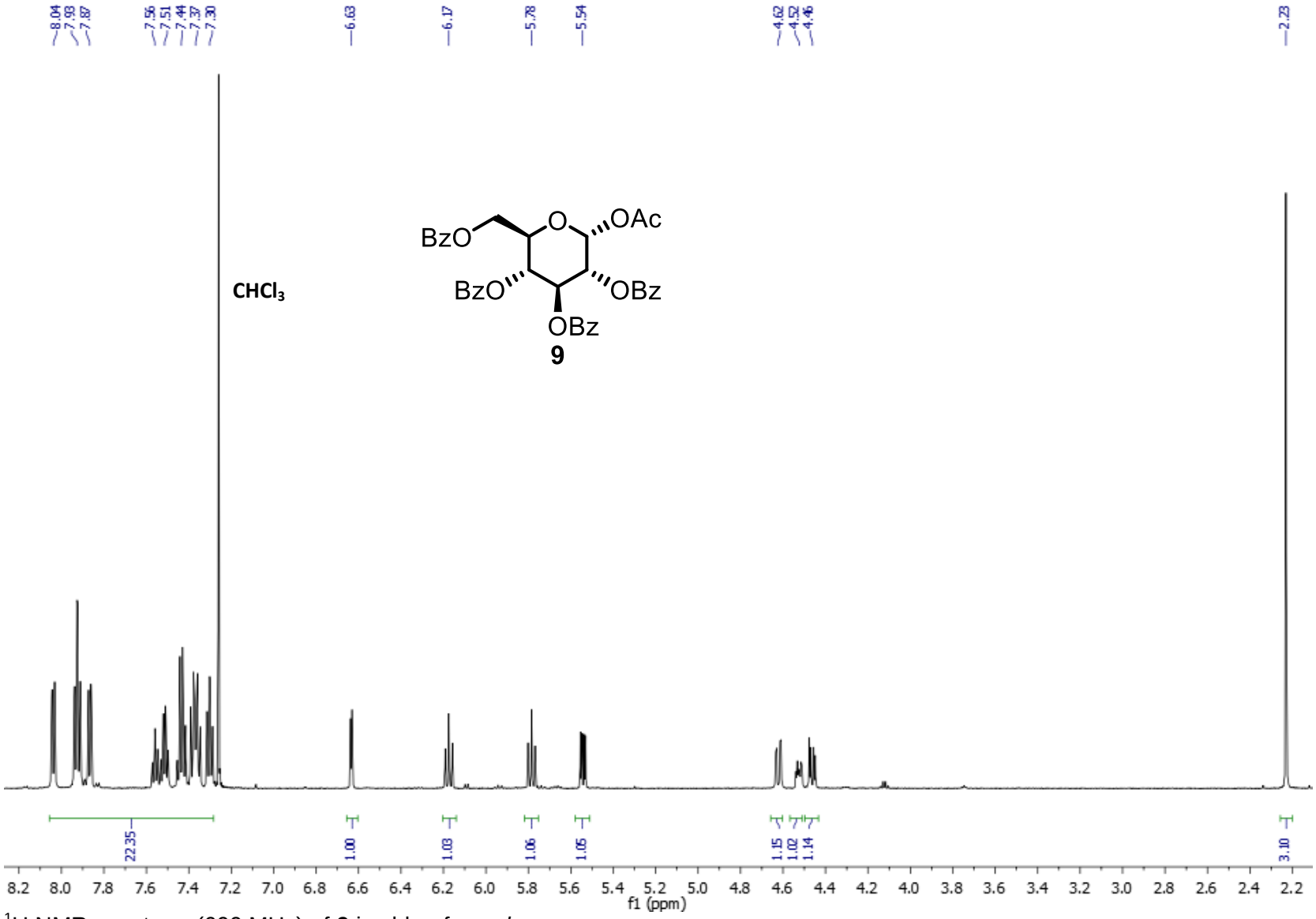
(14) Baer, H. H.; Astles, D. J.; Chin, H.-C.; Siemsen, L. *Can. J. Chem.* **1985**, *63*, 432–439.

(15) Jeong, P. Y.; Jung, M.; Yim, Y. H.; Kim, H.; Park, M.; Hong, E.; Lee, W.; Kim, Y. H.; Kim, K.; Paik, Y. K. *Nature* **2005**, *433*, 541–545.

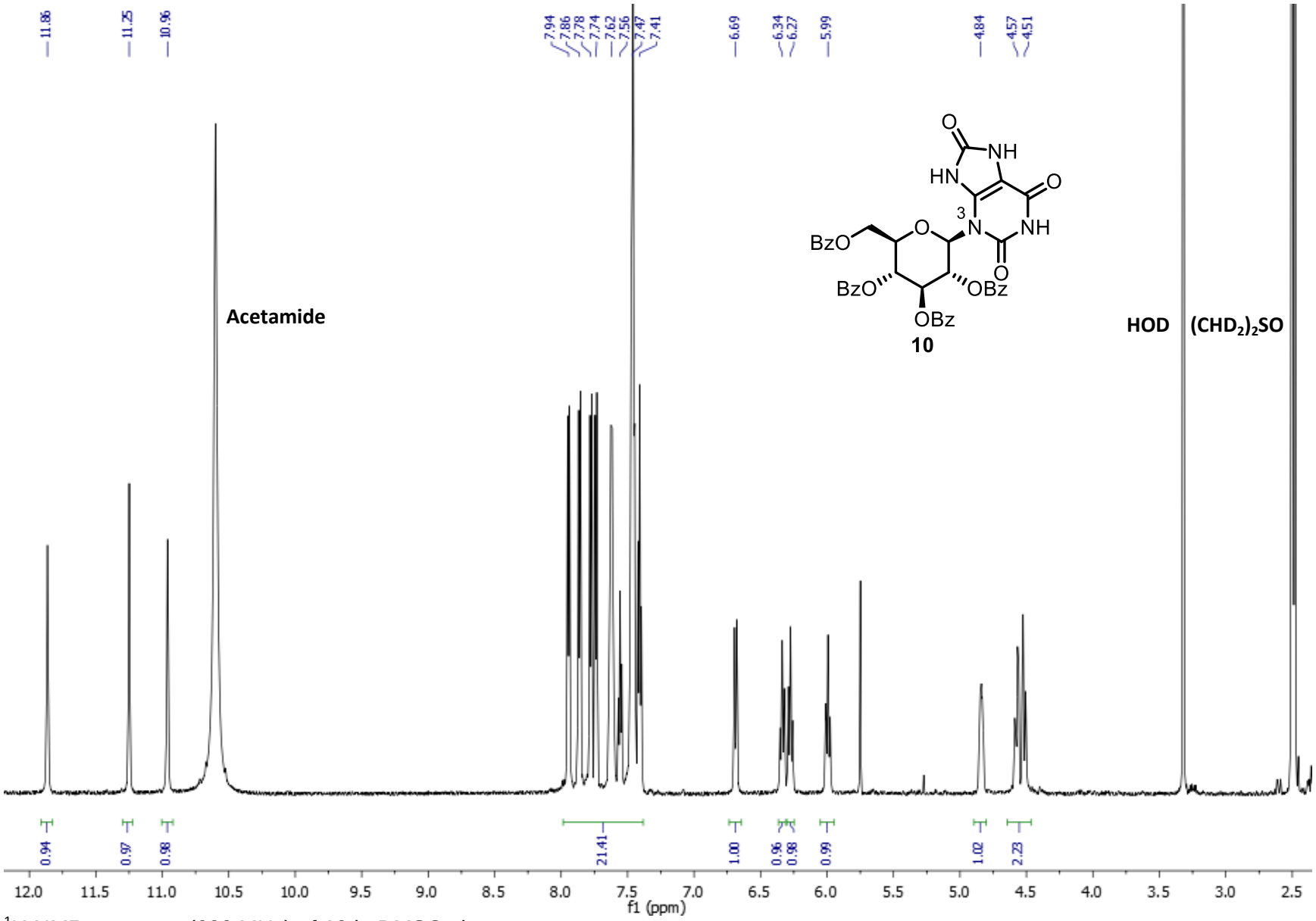
(16) Zhang, Y. K.; Sanchez-Ayala, M. A.; Sternberg, P. W.; Srinivasan, J.; Schroeder, F. C. *Org. Lett.* **2017**, *19*, 2837–2840.

(17) Artyukhin, A. B.; Zhang, Y. K.; Akagi, A. E.; Panda, O.; Sternberg, P. W.; Schroeder, F. C. *J. Am. Chem. Soc.* **2018**, *140*, 2841–2852.

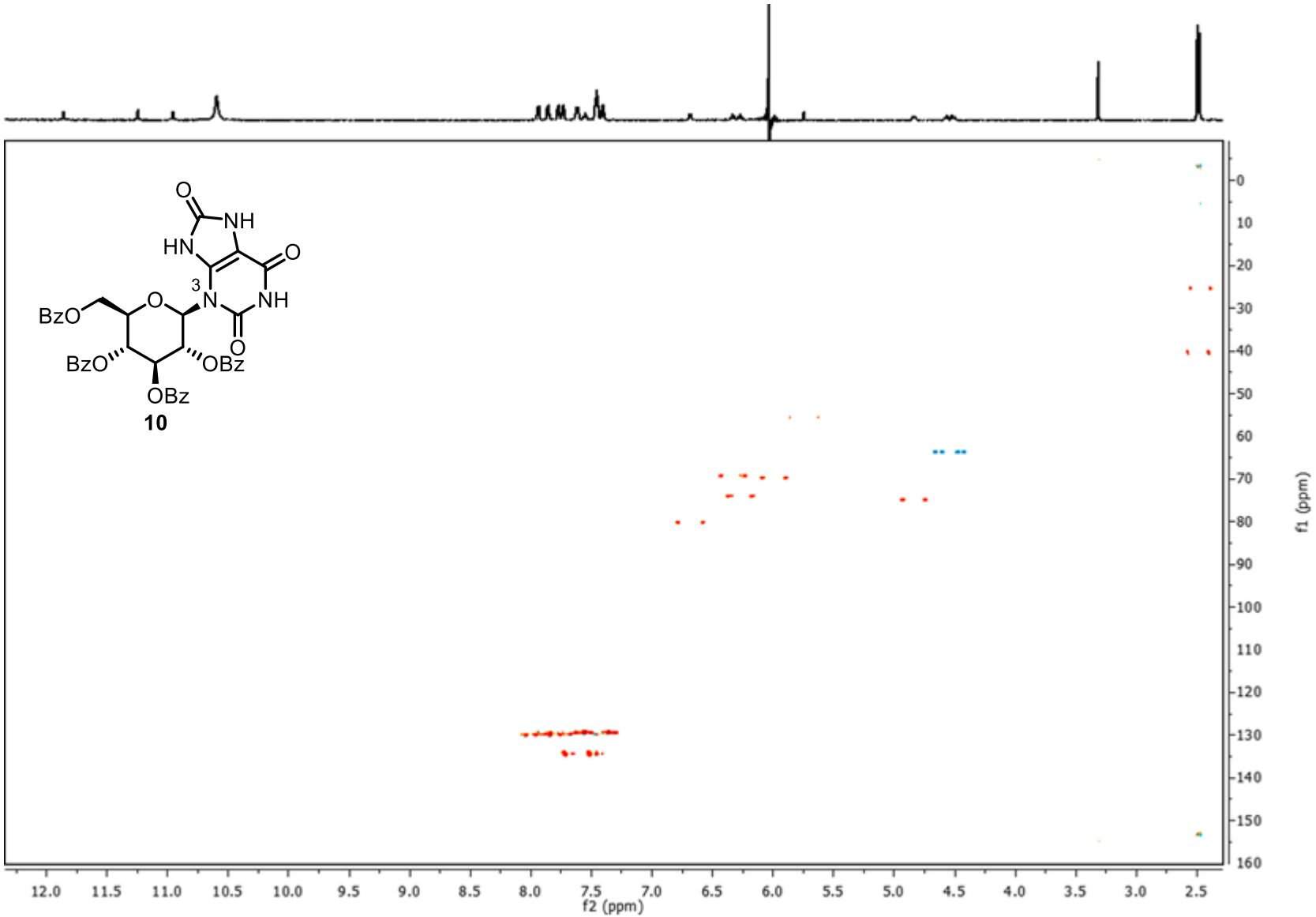
5. NMR spectra appendix



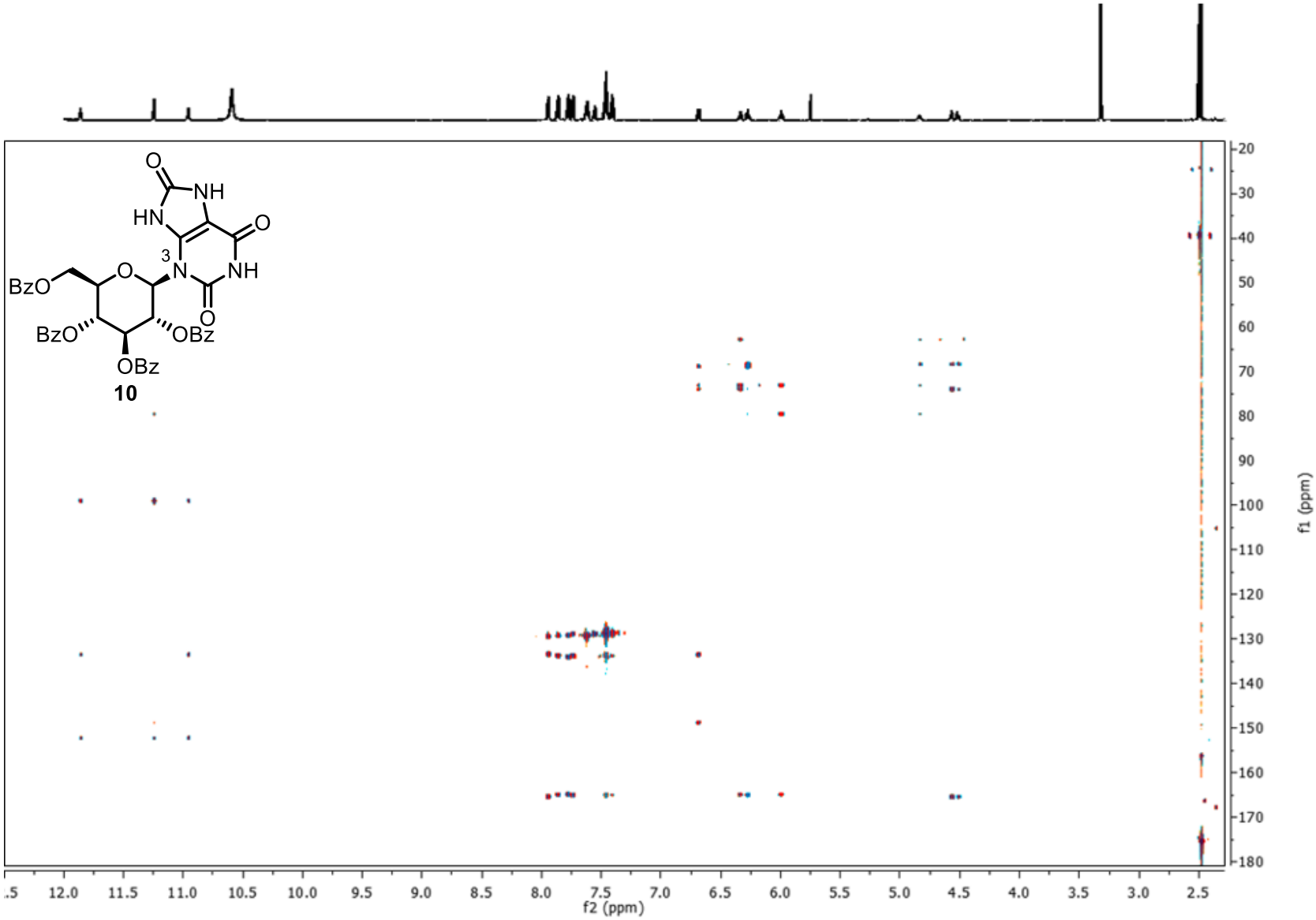
^{1}H NMR spectrum (600 MHz) of **9** in chloroform-*d*.



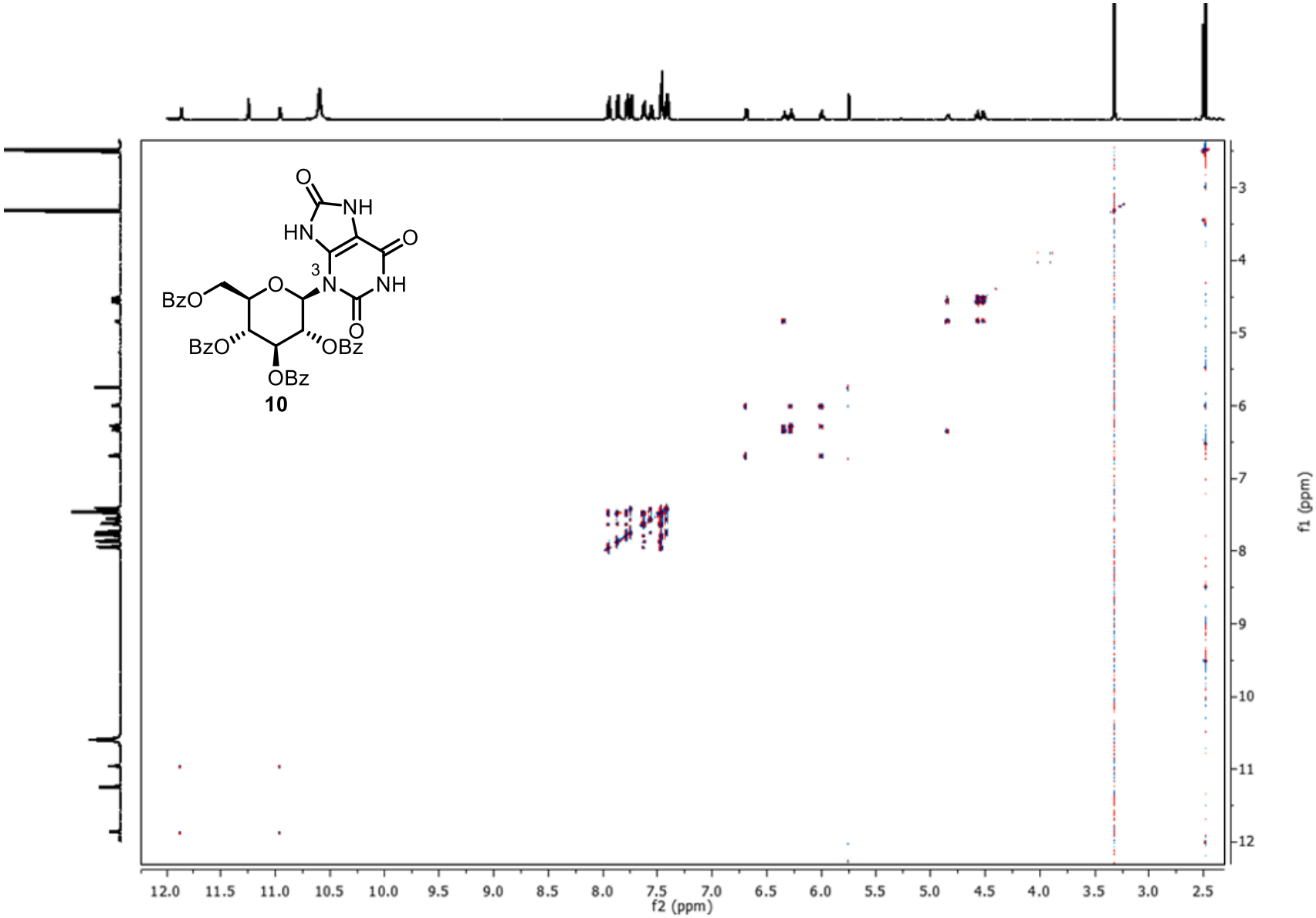
^{1}H NMR spectrum (600 MHz) of **10** in DMSO-d_6.



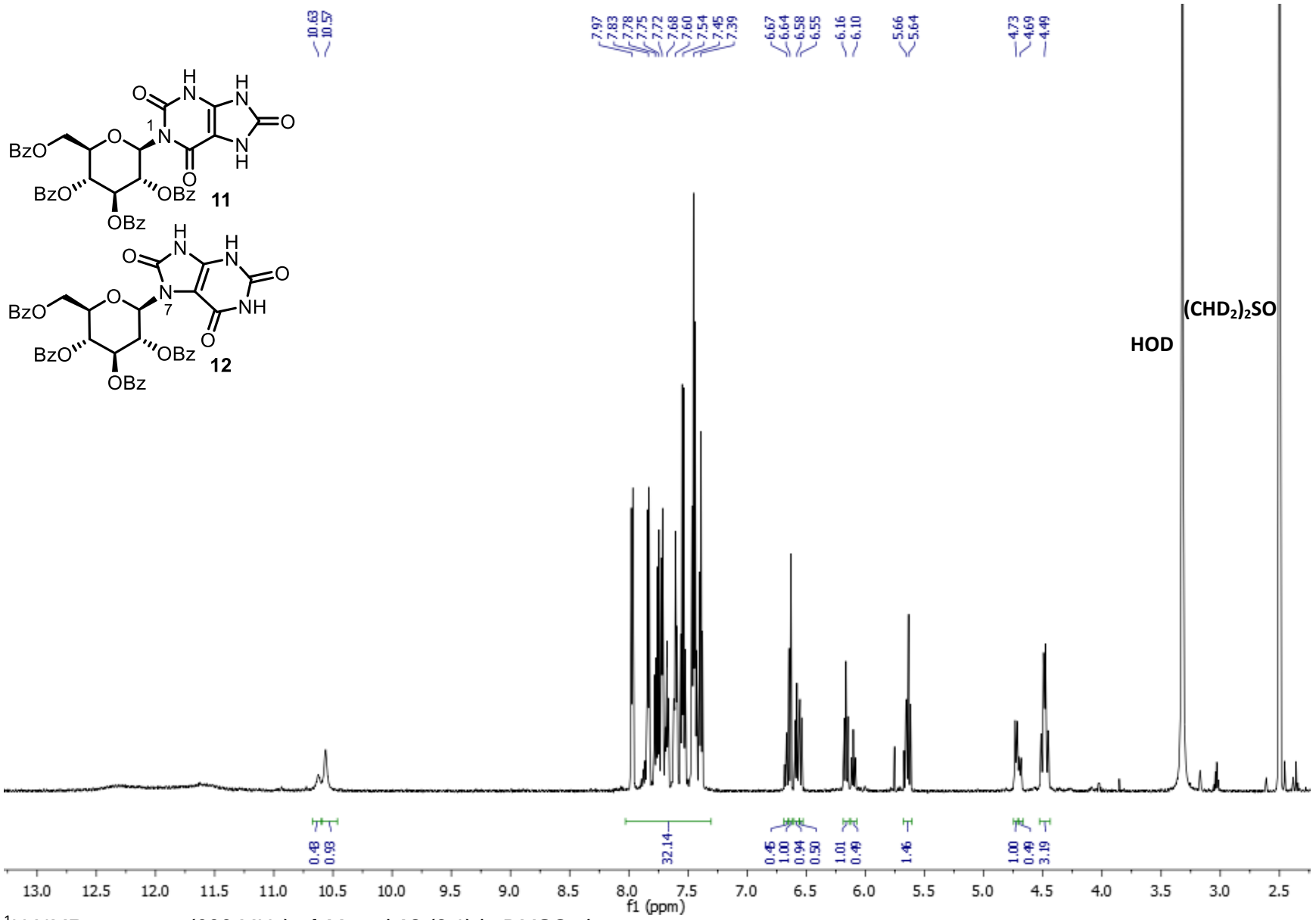
HSQC spectrum (800 MHz) of **10** in DMSO-d_6.



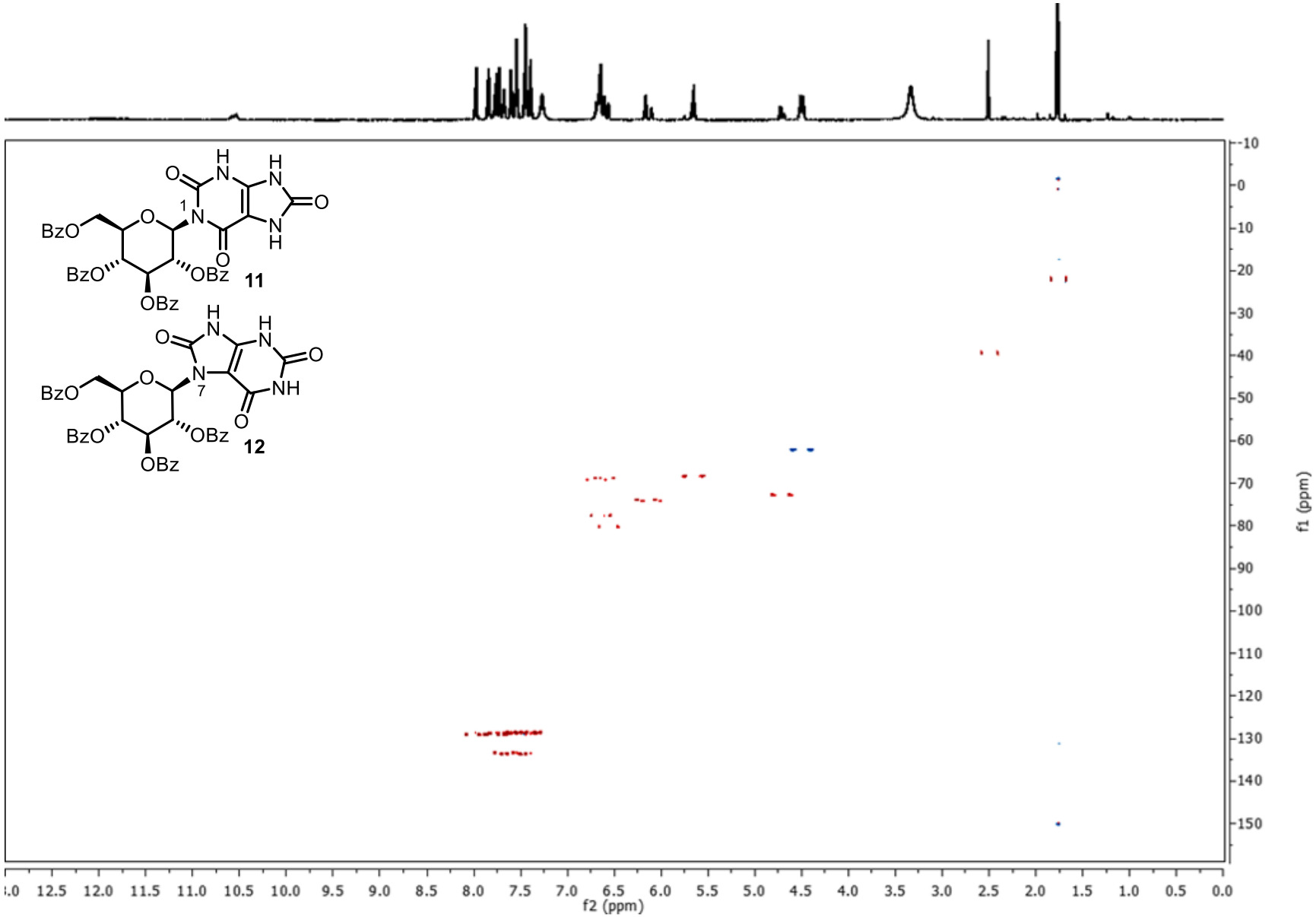
HMBC spectrum (800 MHz) of **10** in DMSO-d_6.



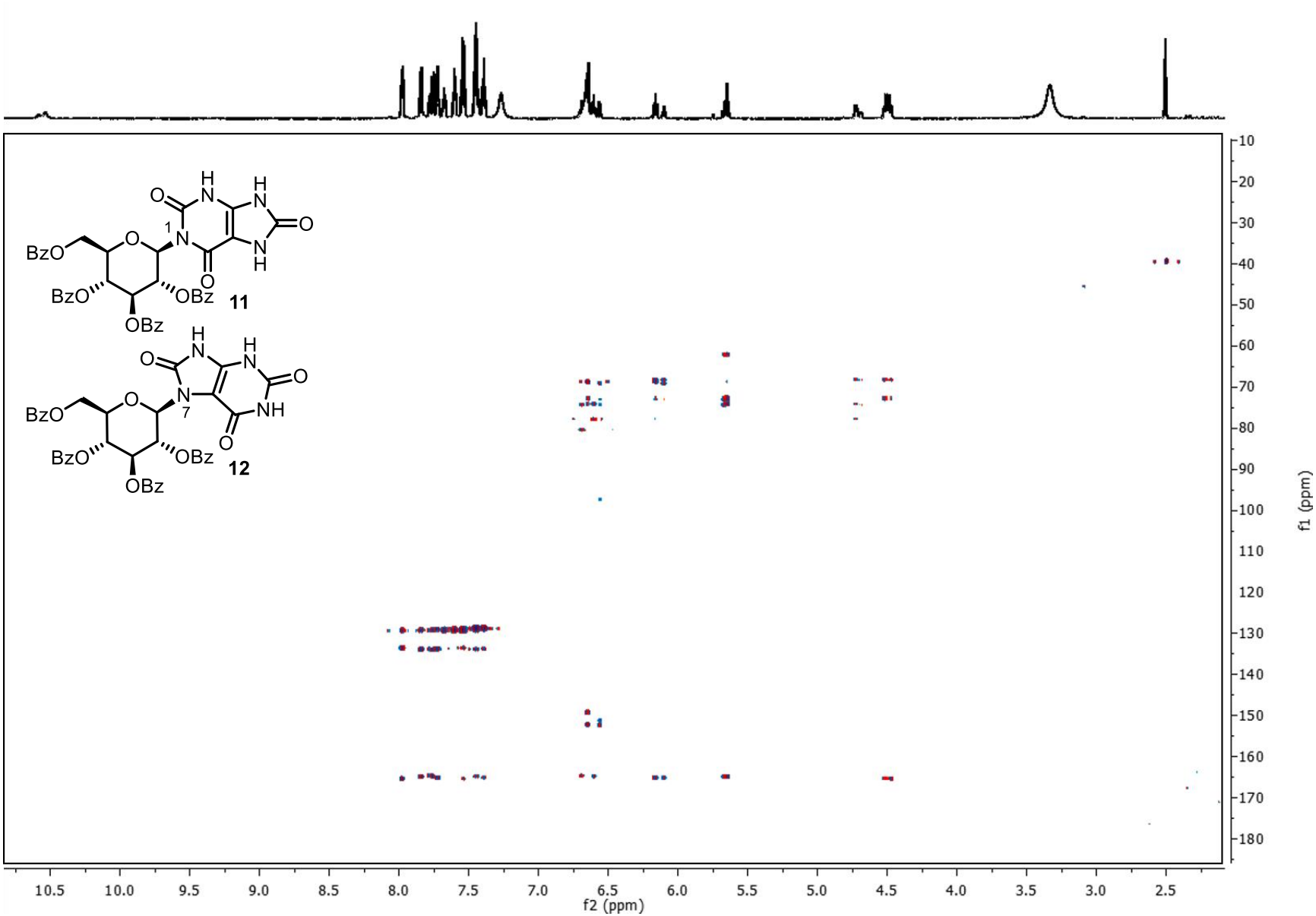
dqfCOSY spectrum (800 MHz) of **10** in DMSO-d_6.



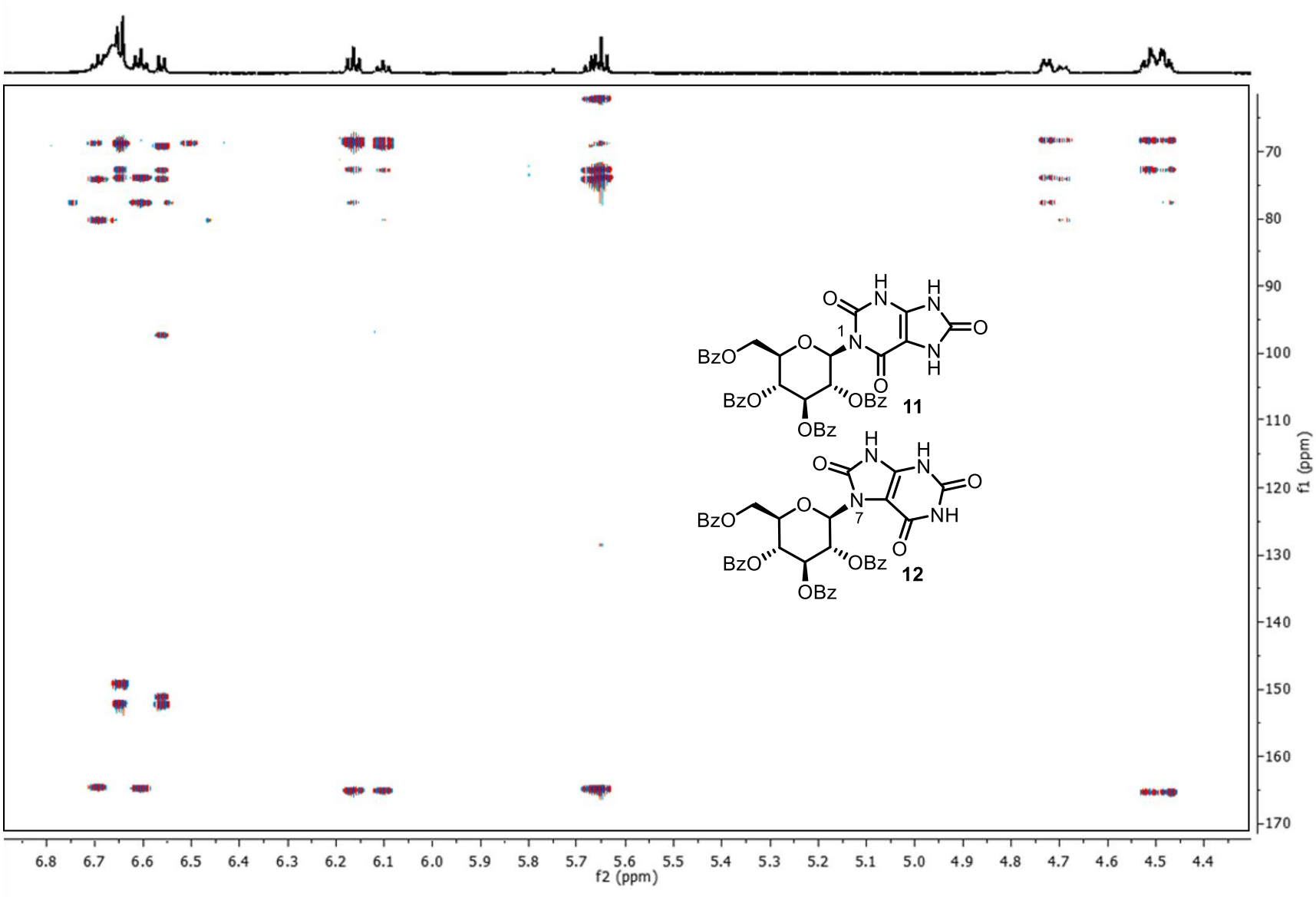
^{1}H NMR spectrum (600 MHz) of **11** and **12** (2:1) in DMSO-d_6.



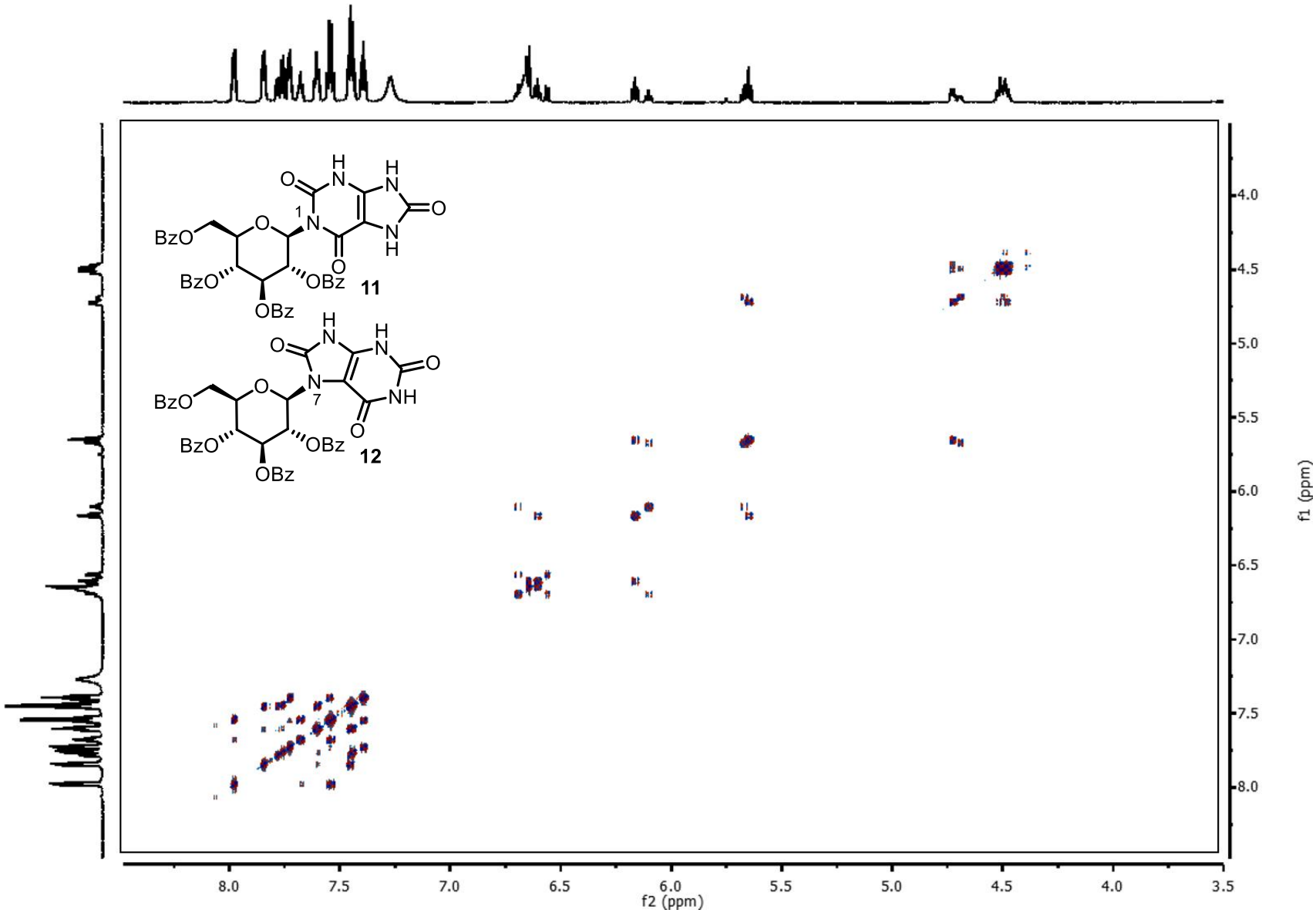
HSQC spectrum (800 MHz) of **11** and **12** (2:1) in DMSO-d_6.



HMBC spectrum (800 MHz) of **11** and **12** (2:1) in DMSO-d_6.



Section of HMBC spectrum of **11** and **12** (2:1) in DMSO-d_6.



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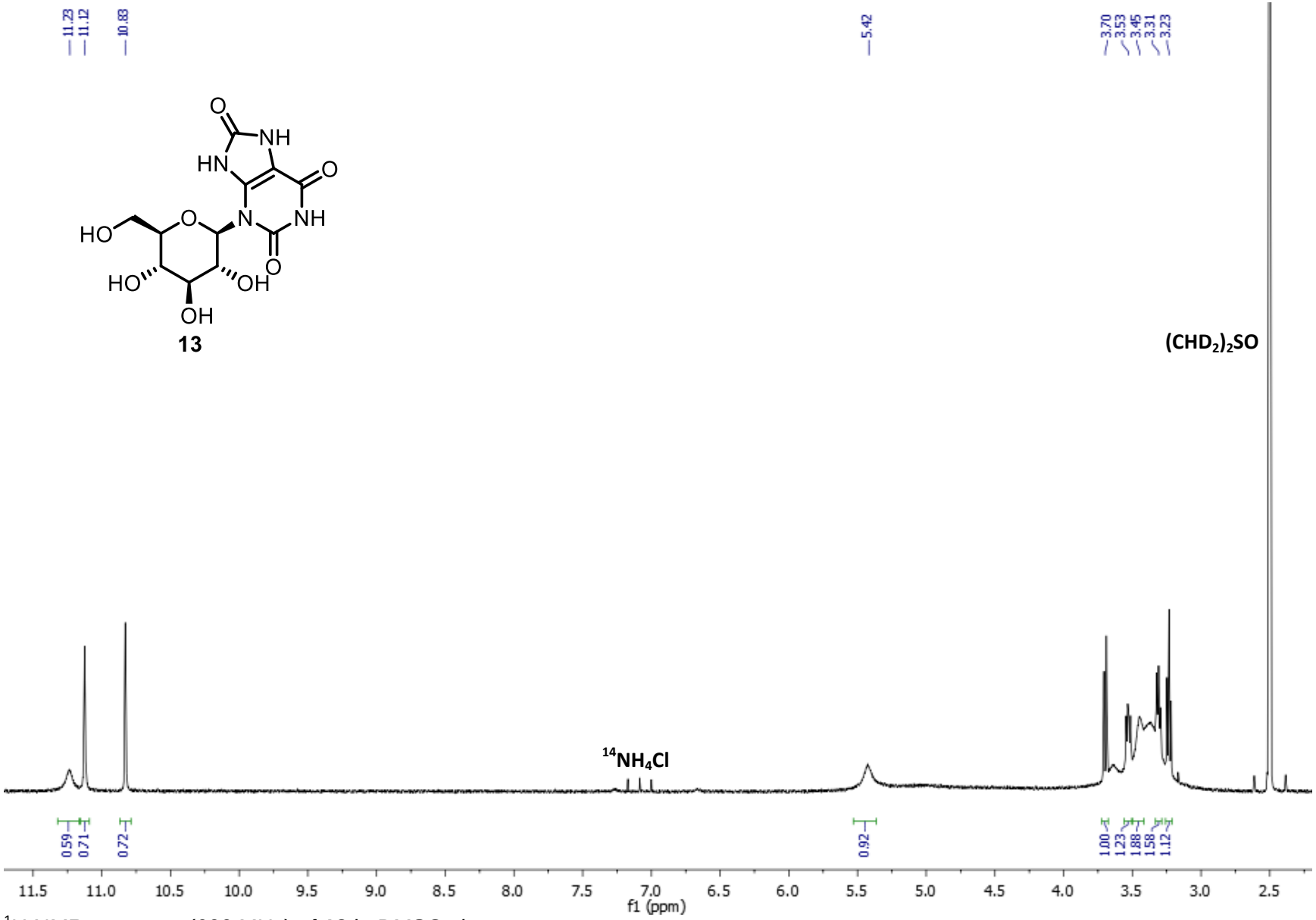
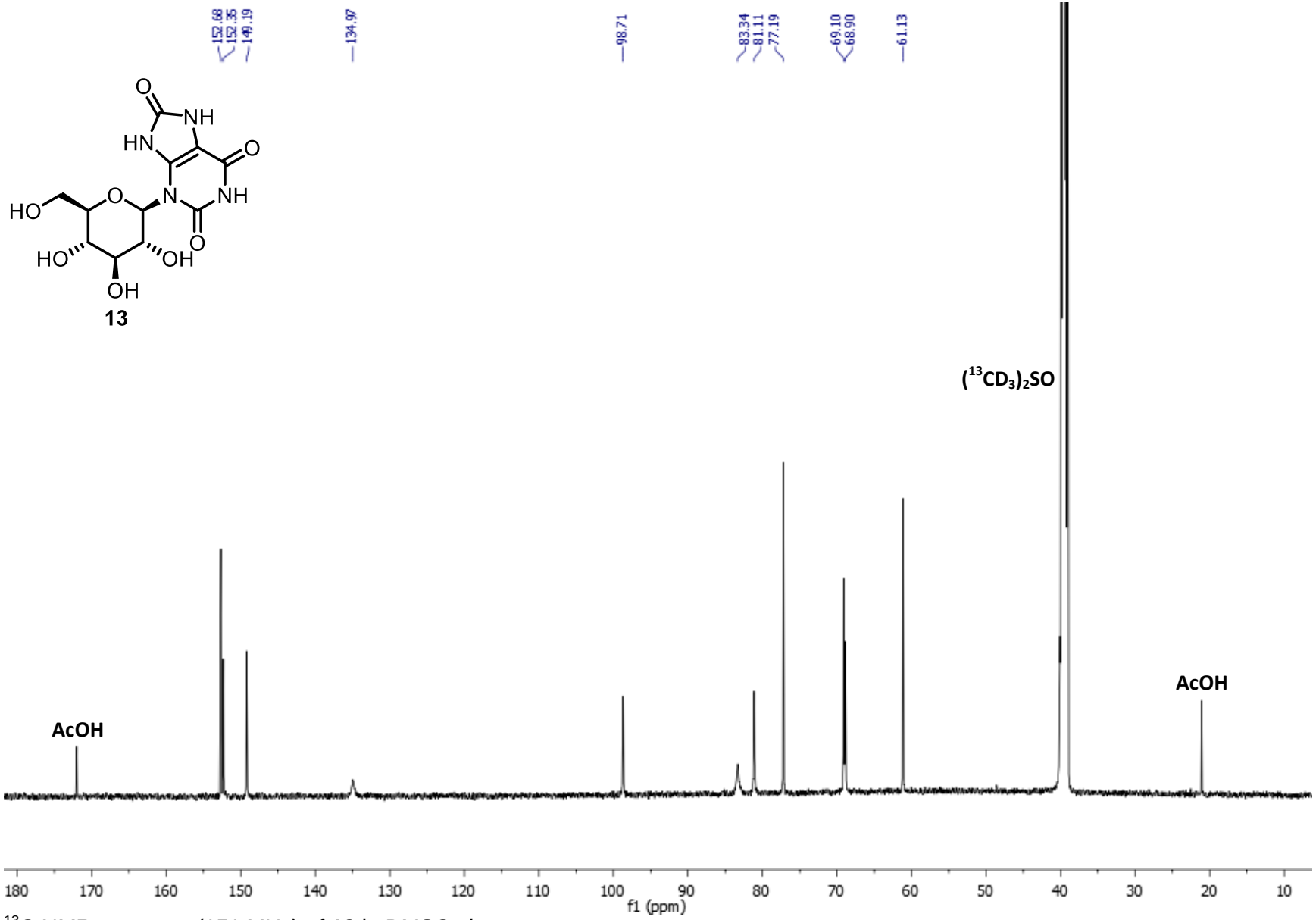
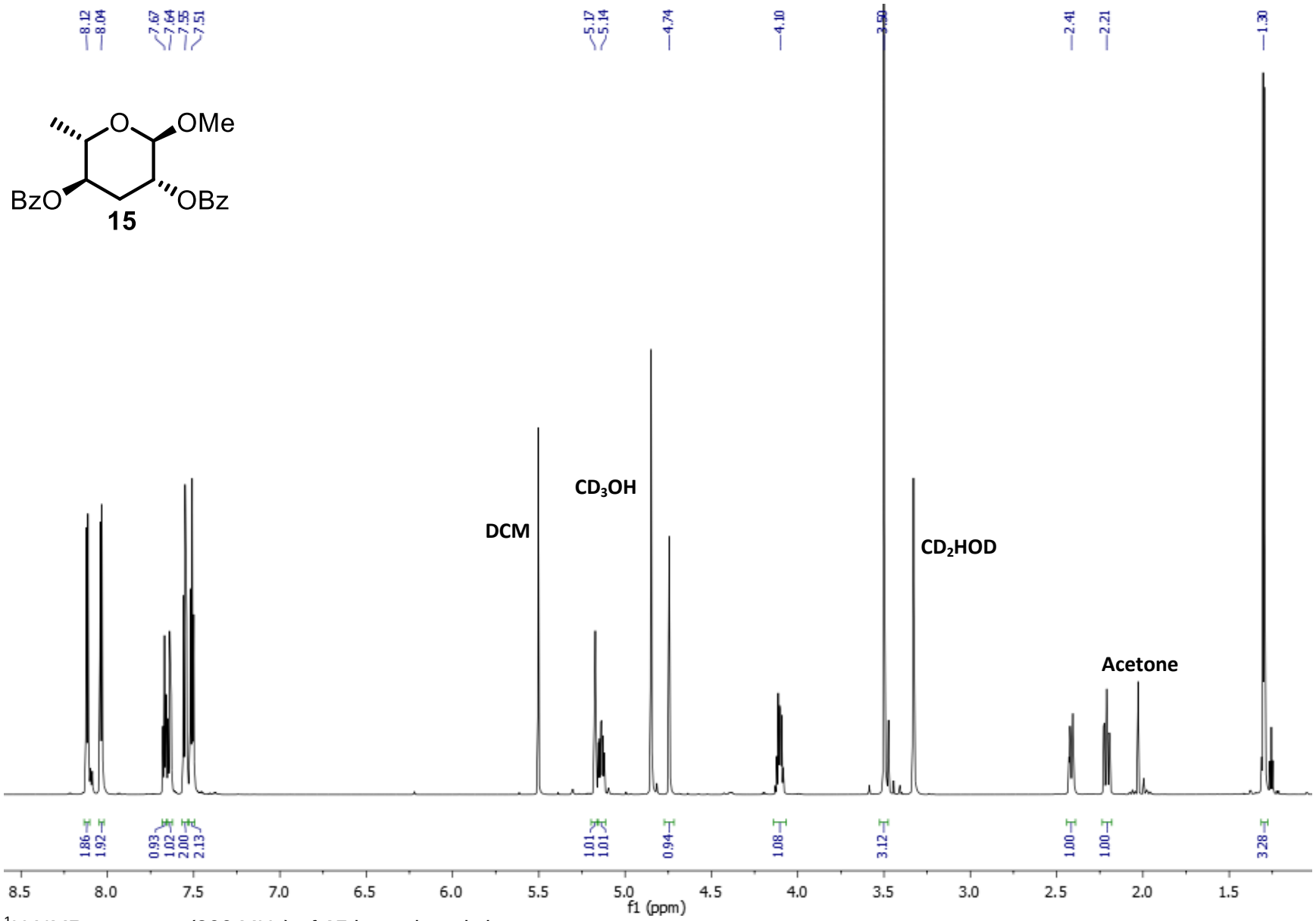


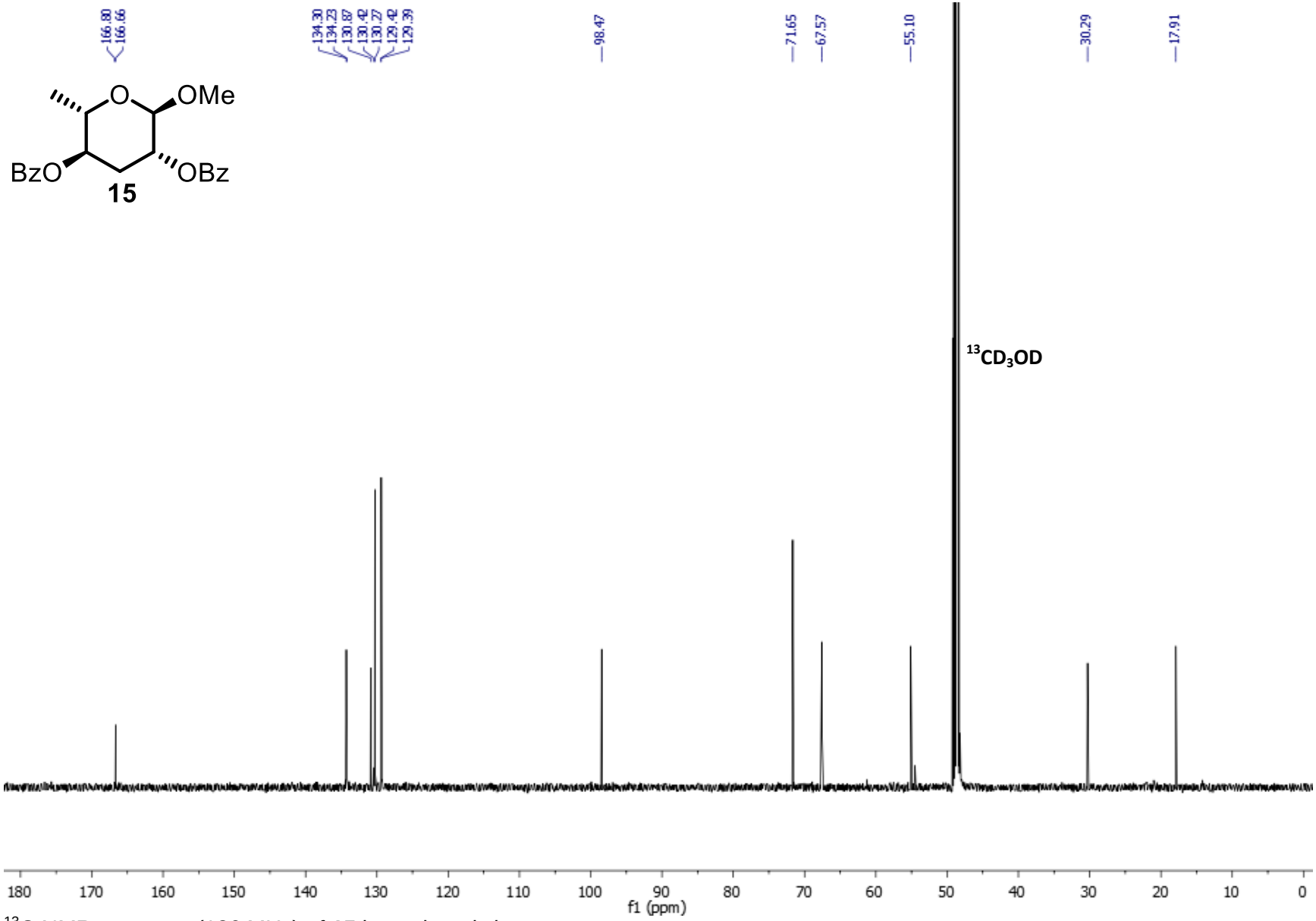
^{1}H NMR spectrum (600 MHz) of **13** in DMSO-d_6.



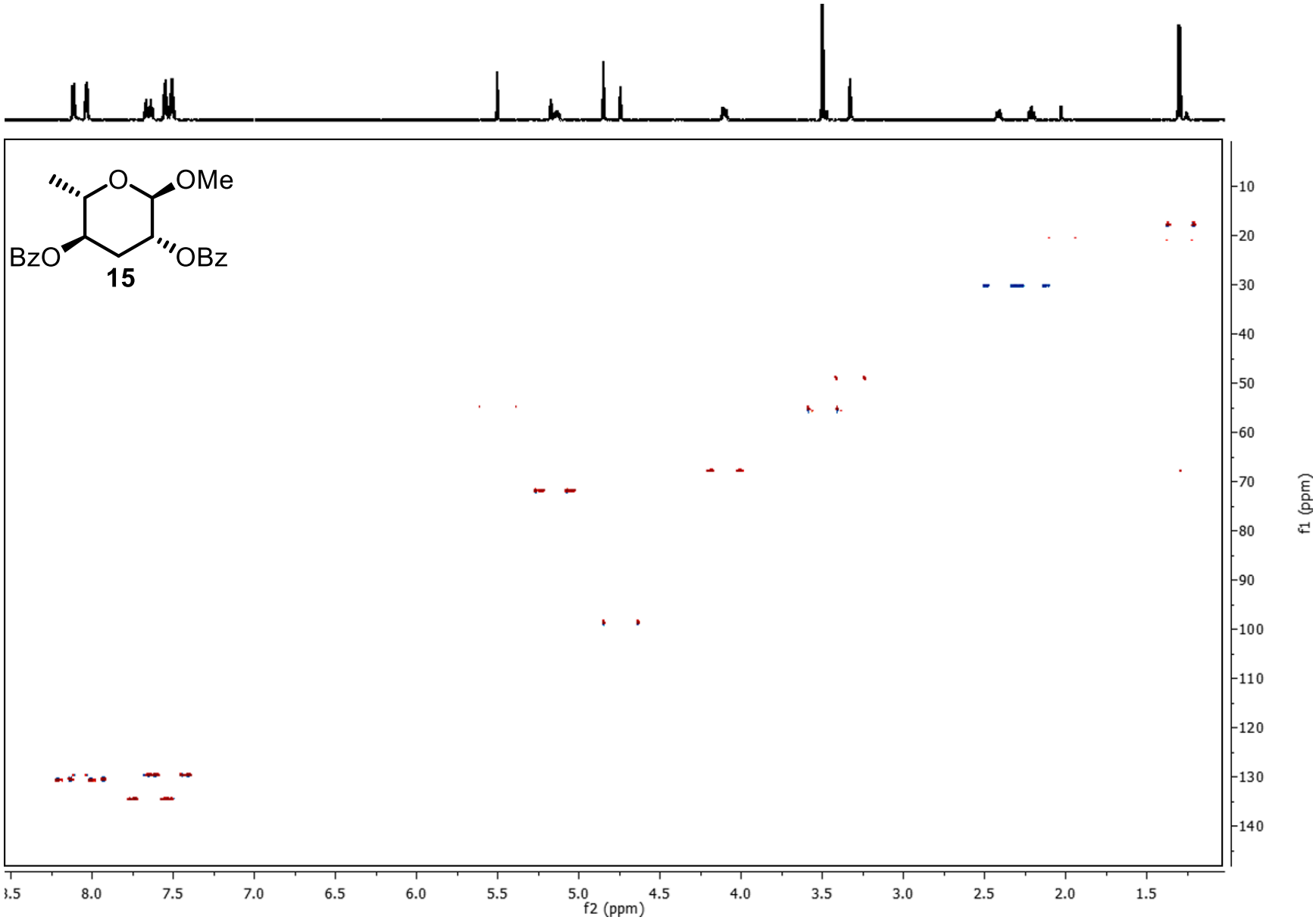
^{13}C NMR spectrum (151 MHz) of **13** in DMSO-d_6.



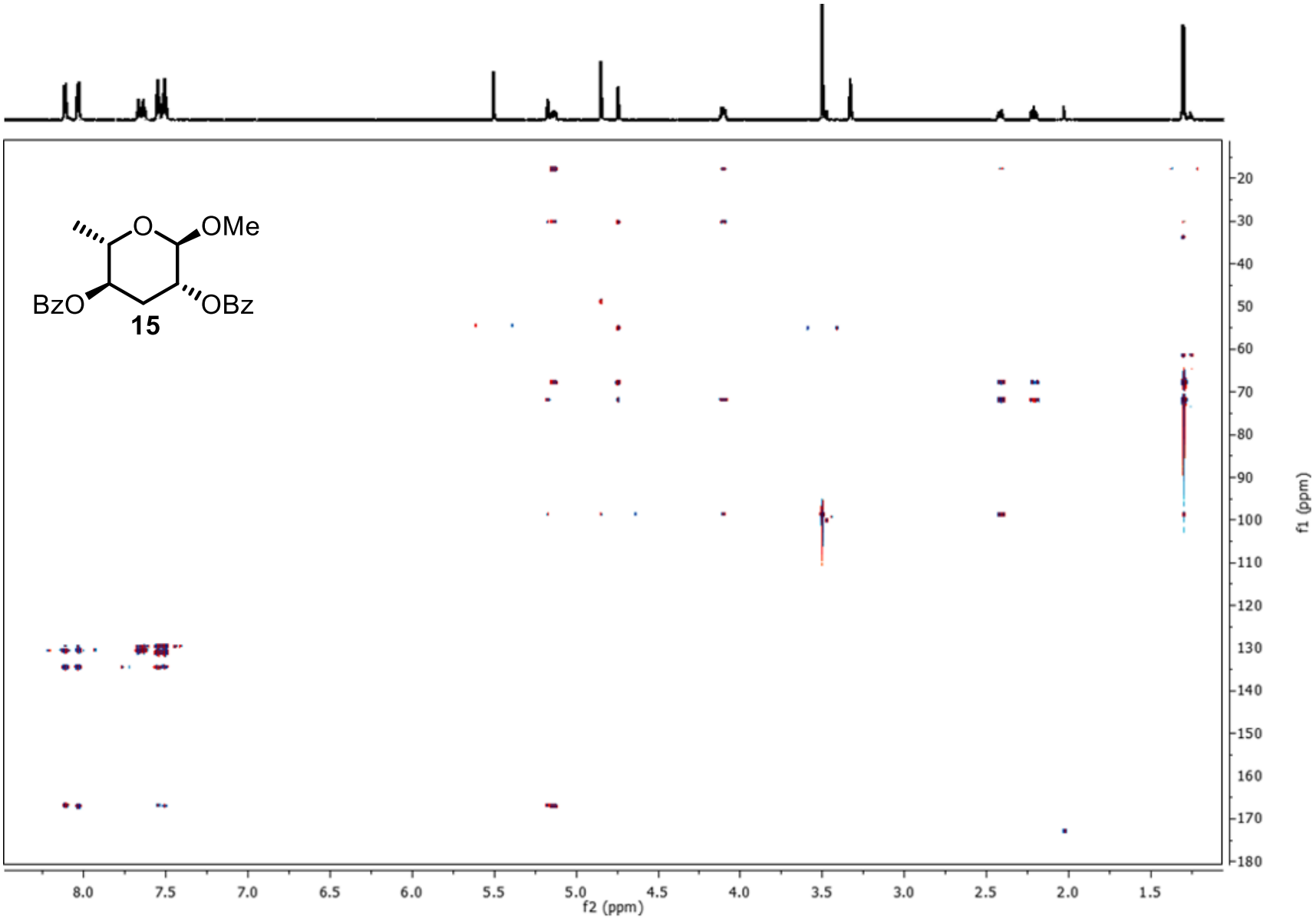
^{1}H NMR spectrum (800 MHz) of **15** in methanol-d_4.



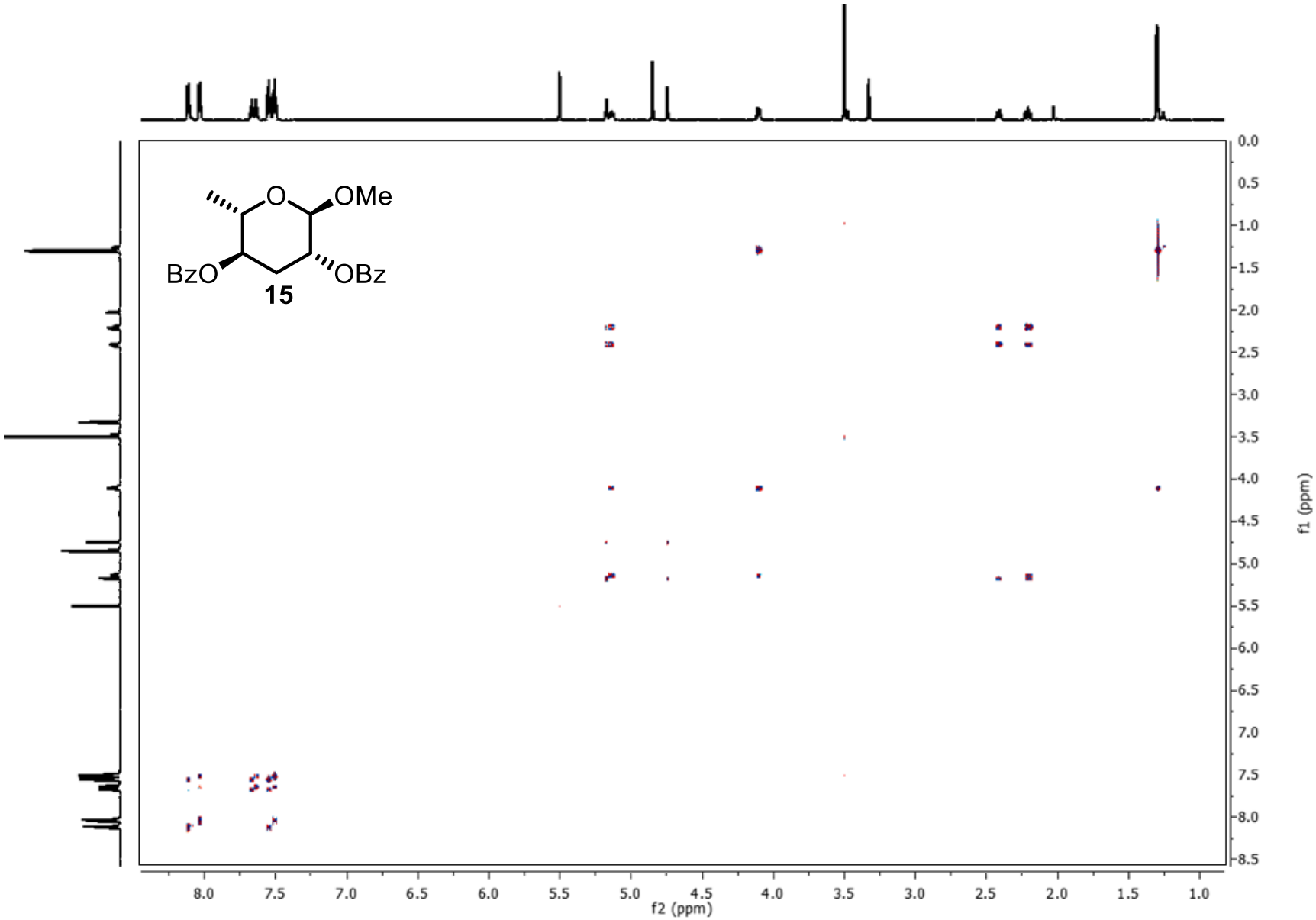
^{13}C NMR spectrum (126 MHz) of **15** in methanol-d_4.



HSQC spectrum (800 MHz) of **15** in methanol-d_4.



HMBC spectrum (800 MHz) of **15** in methanol-d_4.



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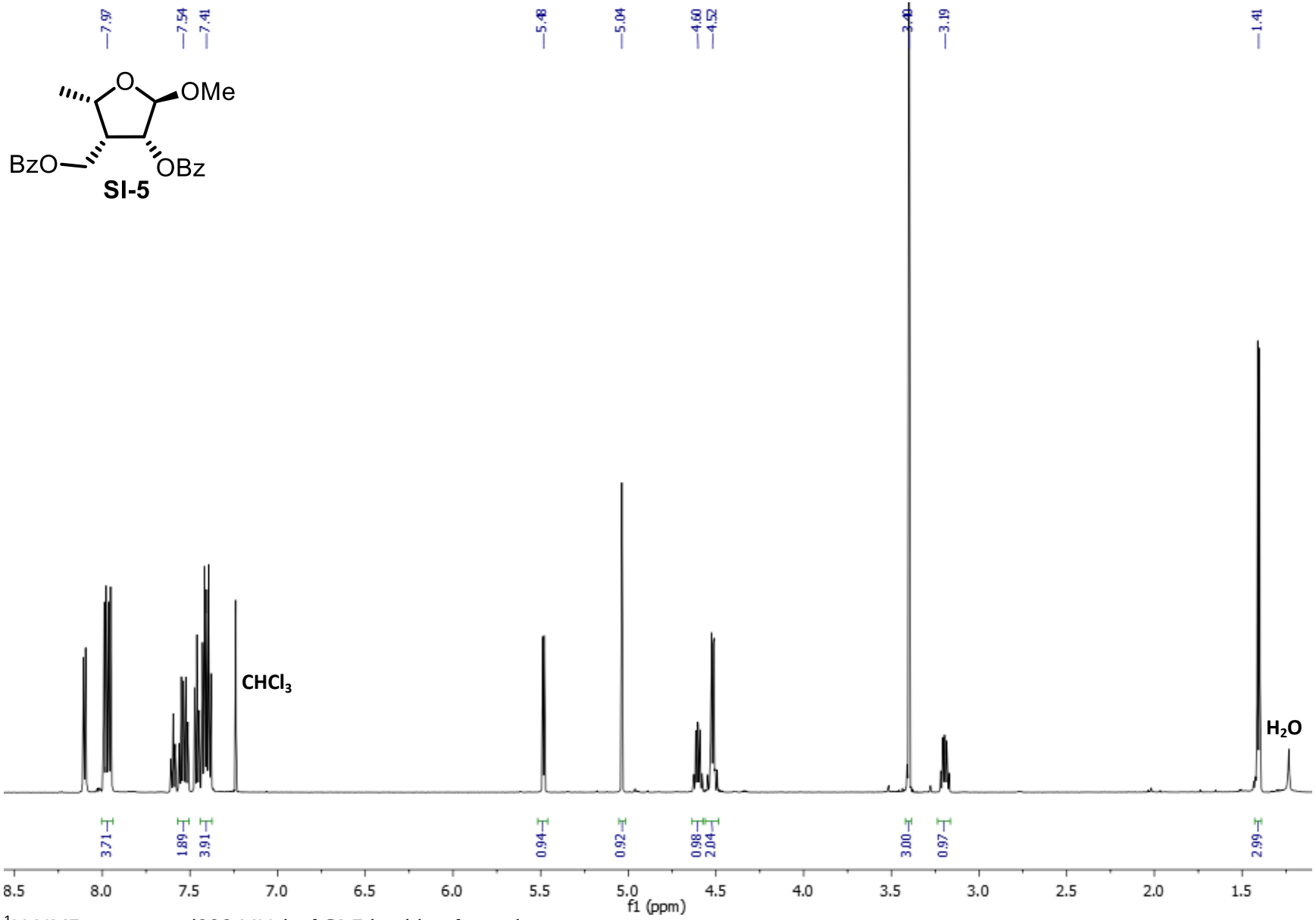
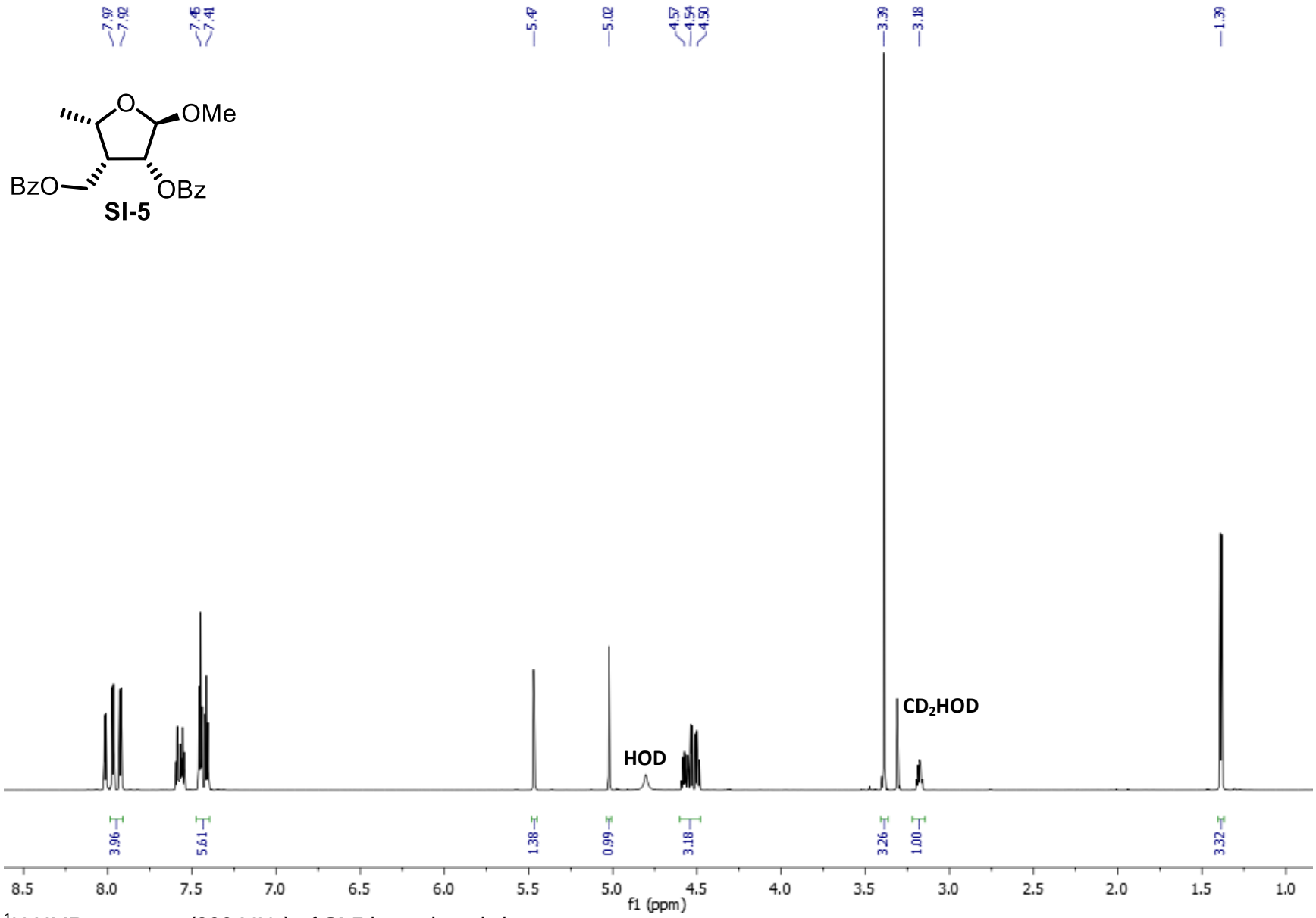
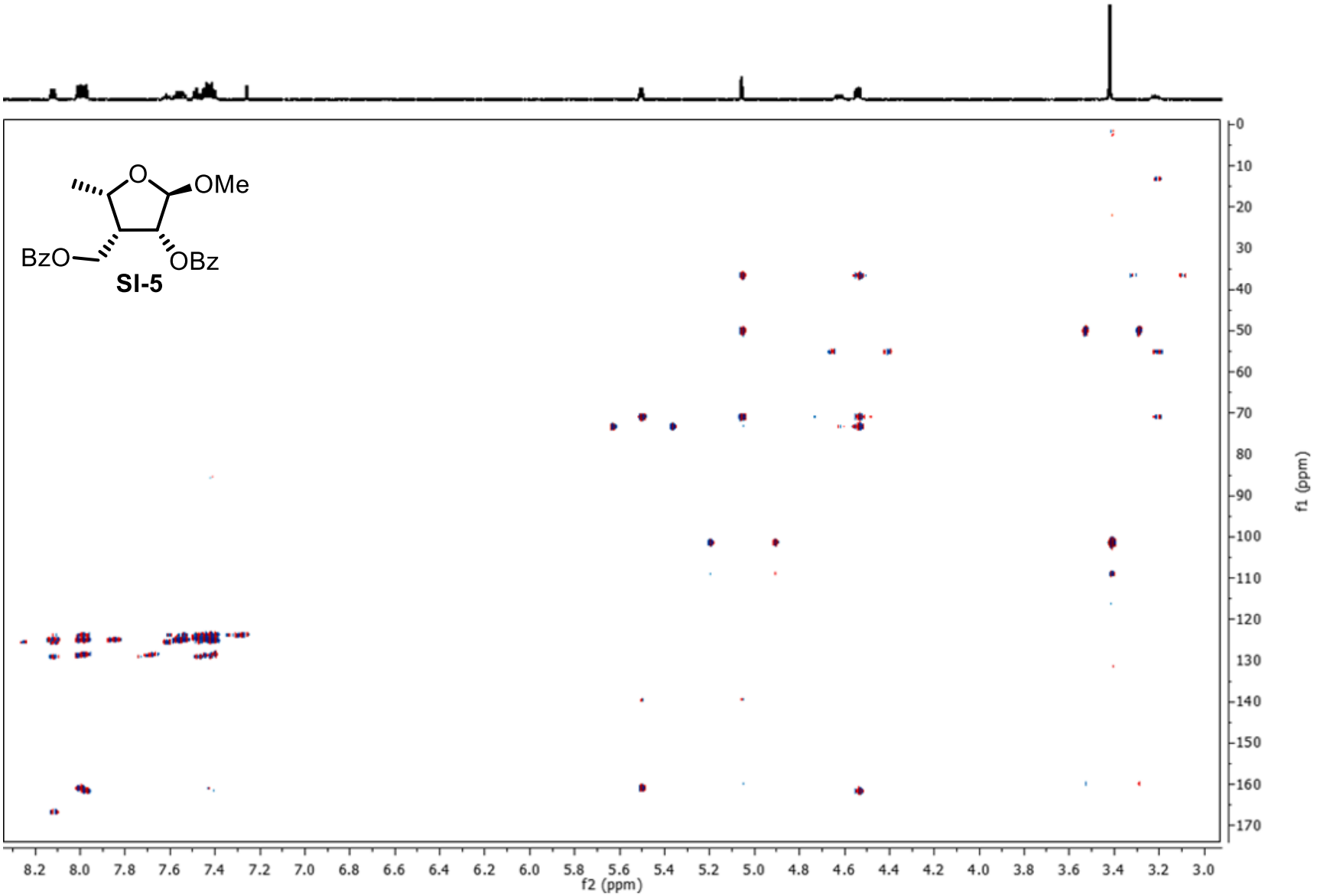


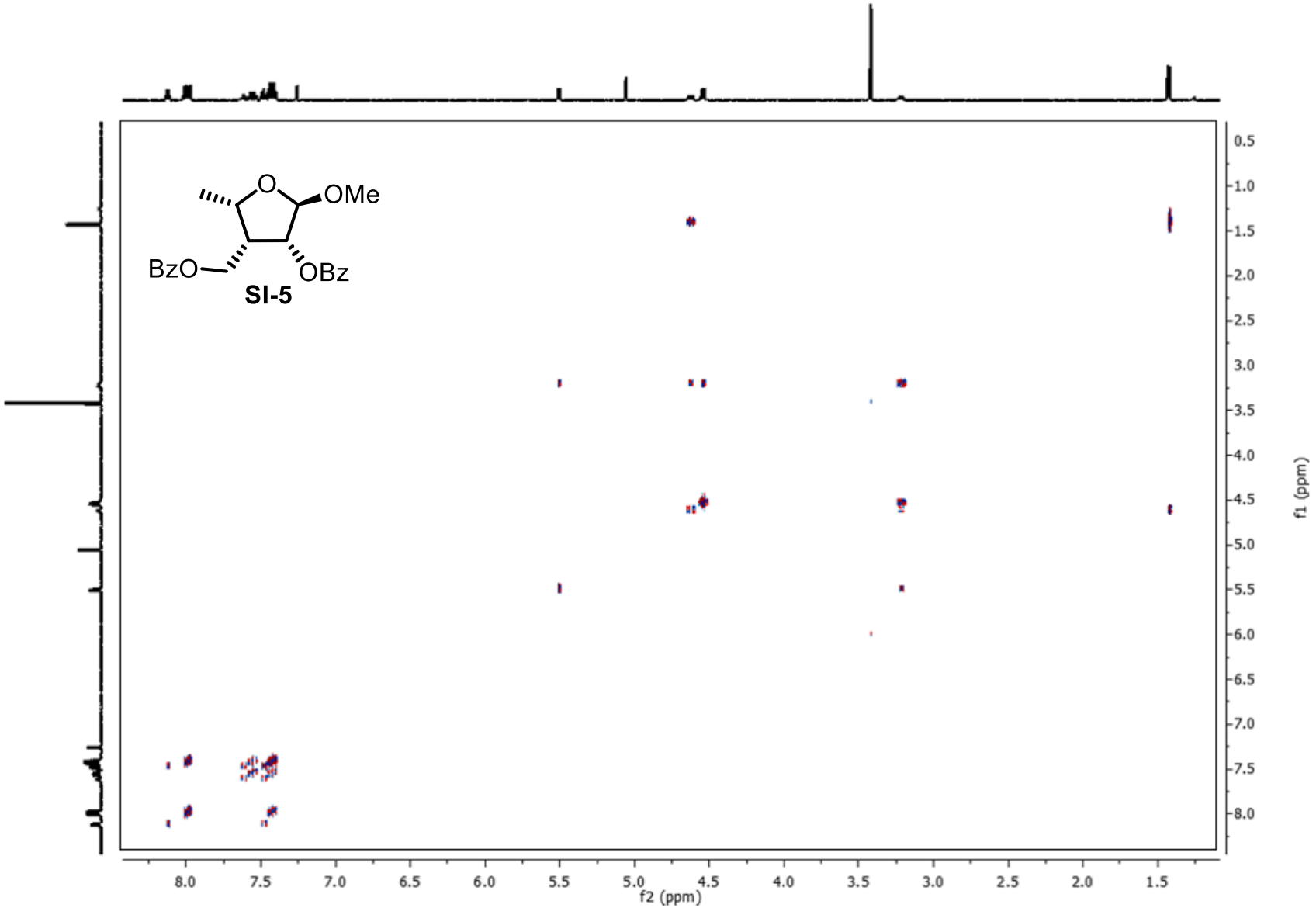
^{1}H NMR spectrum (600 MHz) of **SI-5** in chloroform-*d*.



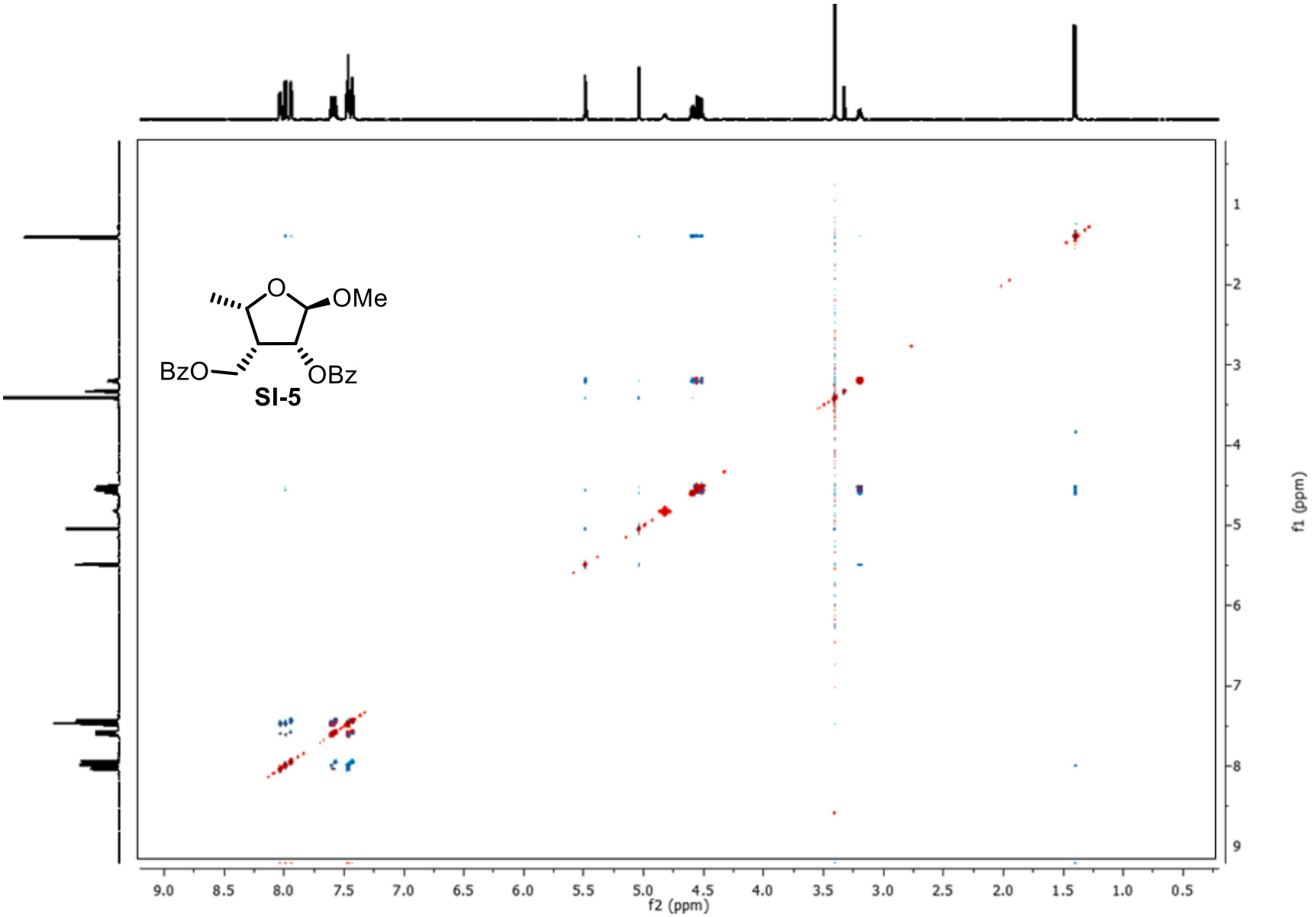
^{1}H NMR spectrum (800 MHz) of **SI-5** in methanol-d_4.



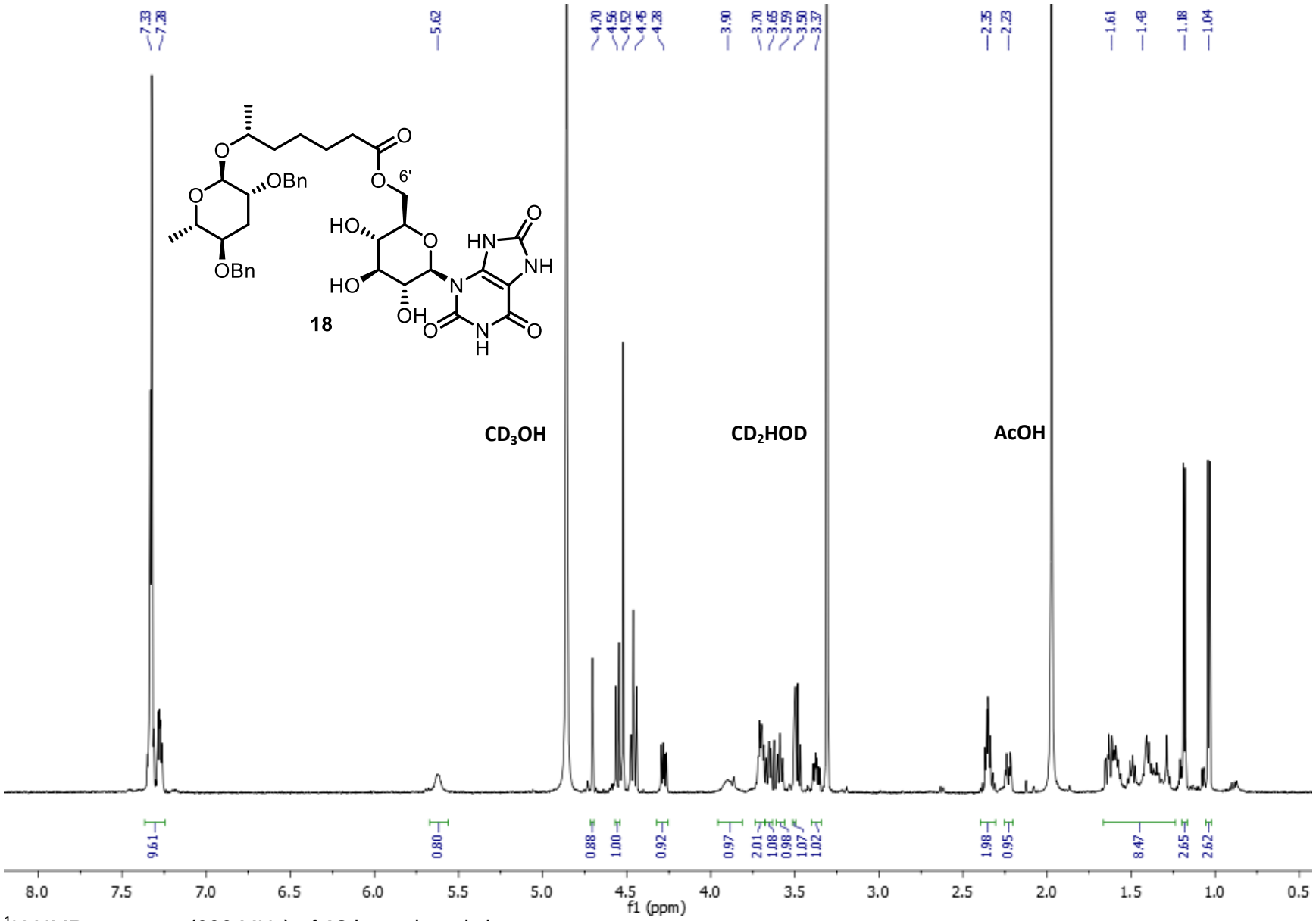
HMBC spectrum (600 MHz) of **SI-5** in chloroform-*d*.



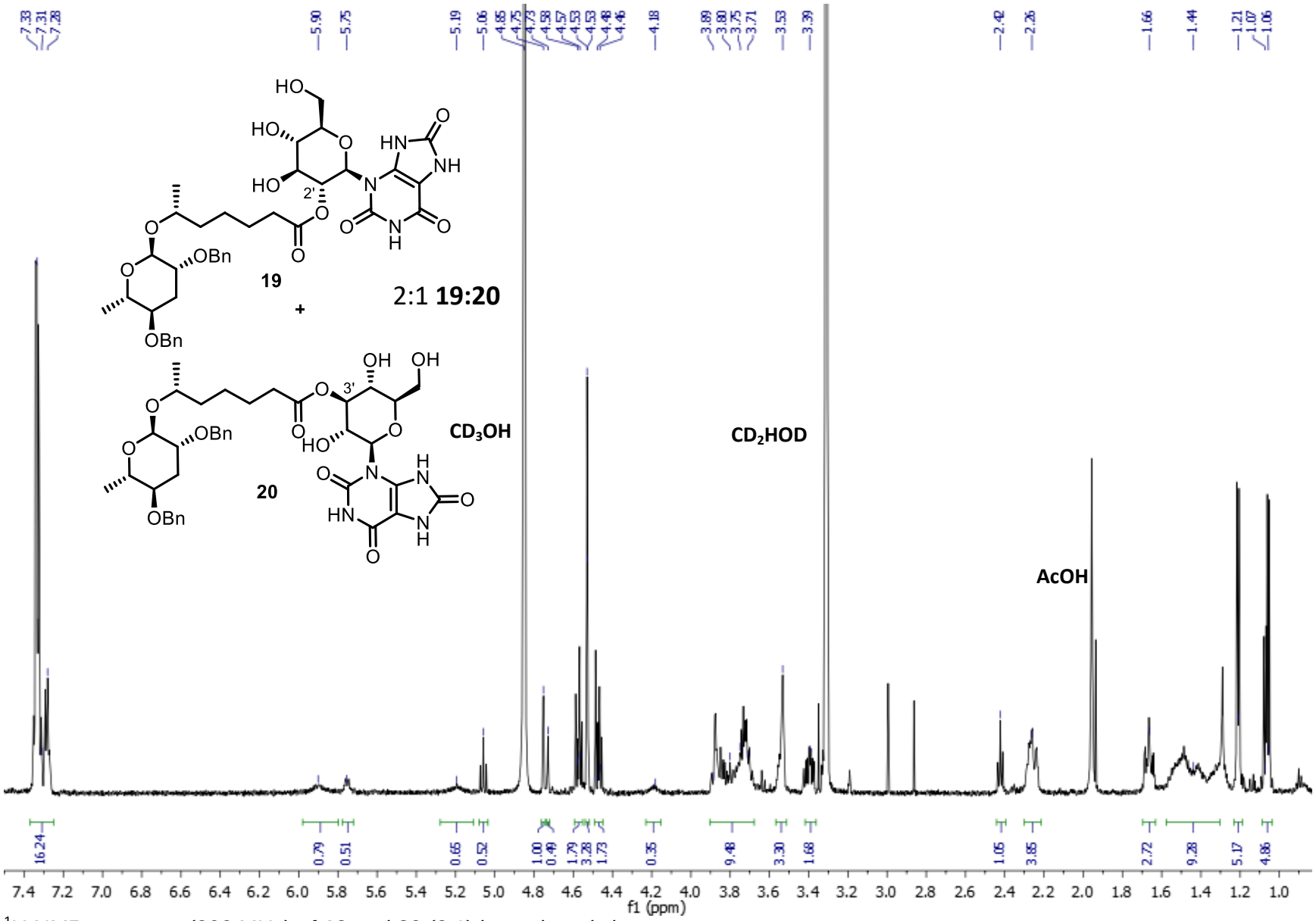
dqfCOSY spectrum (600 MHz) of **SI-5** in chloroform-*d*.



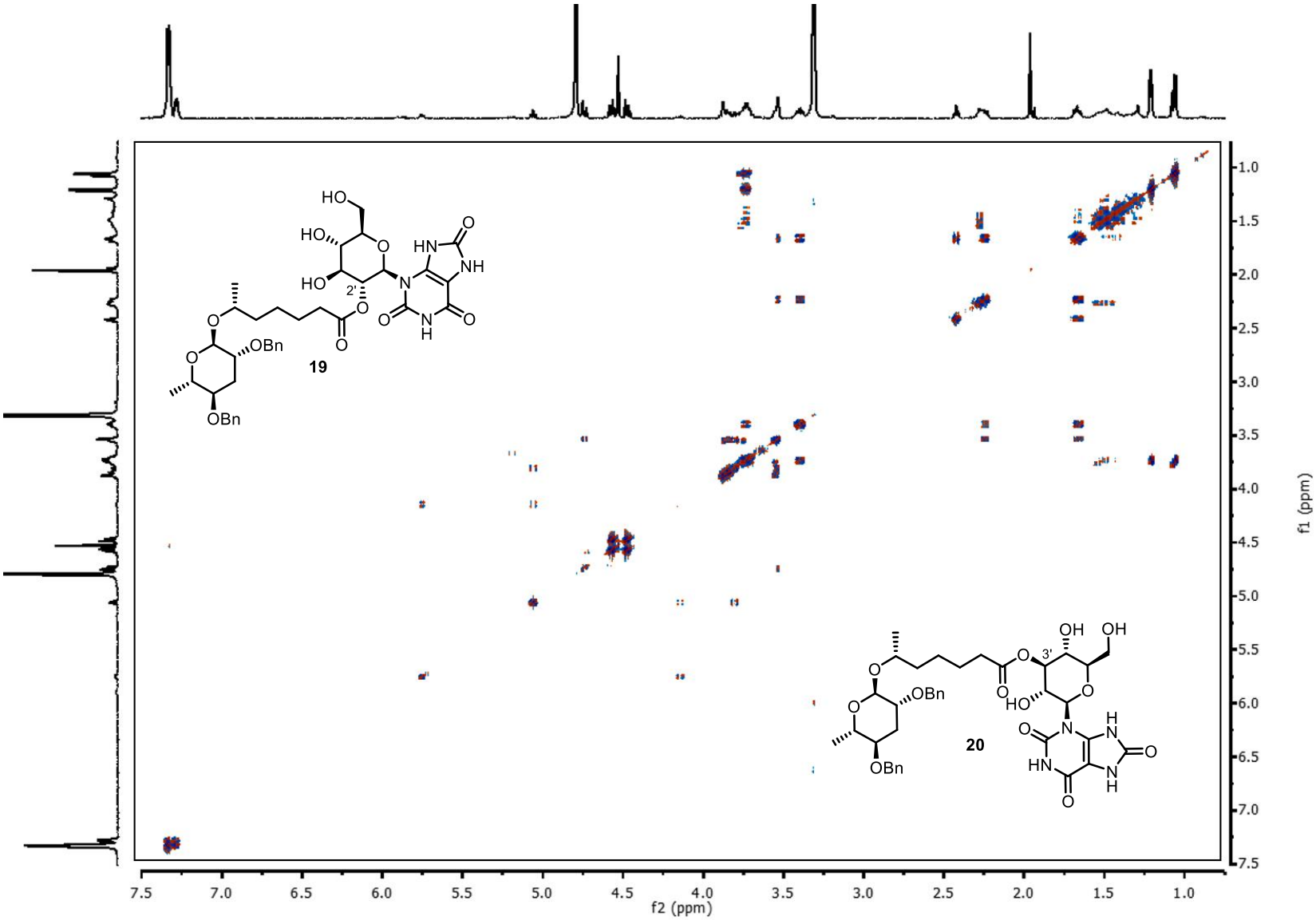
NOESY spectrum (800 MHz) of **SI-5** in methanol-d_4.



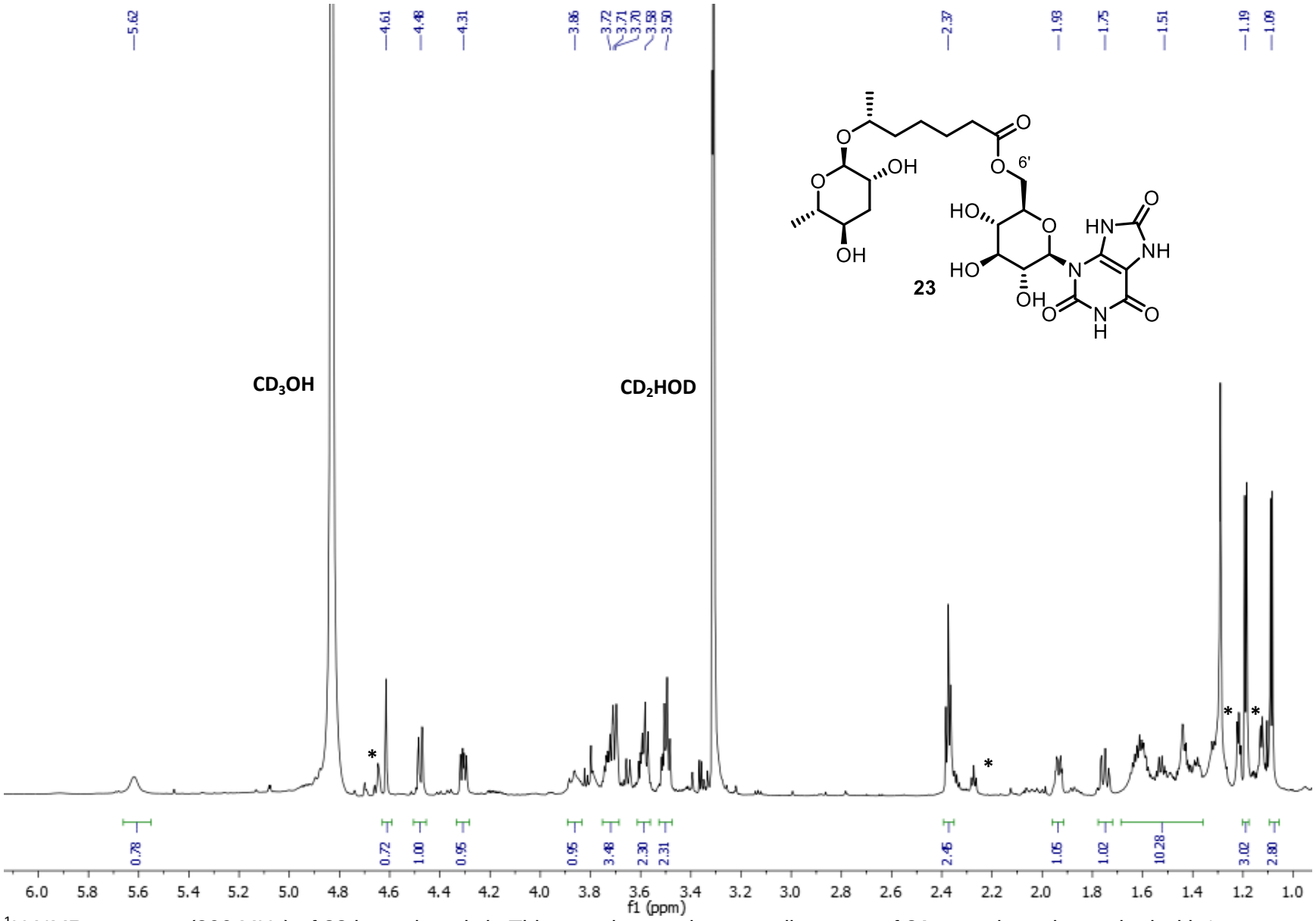
^{1}H NMR spectrum (600 MHz) of **18** in methanol-d_4.



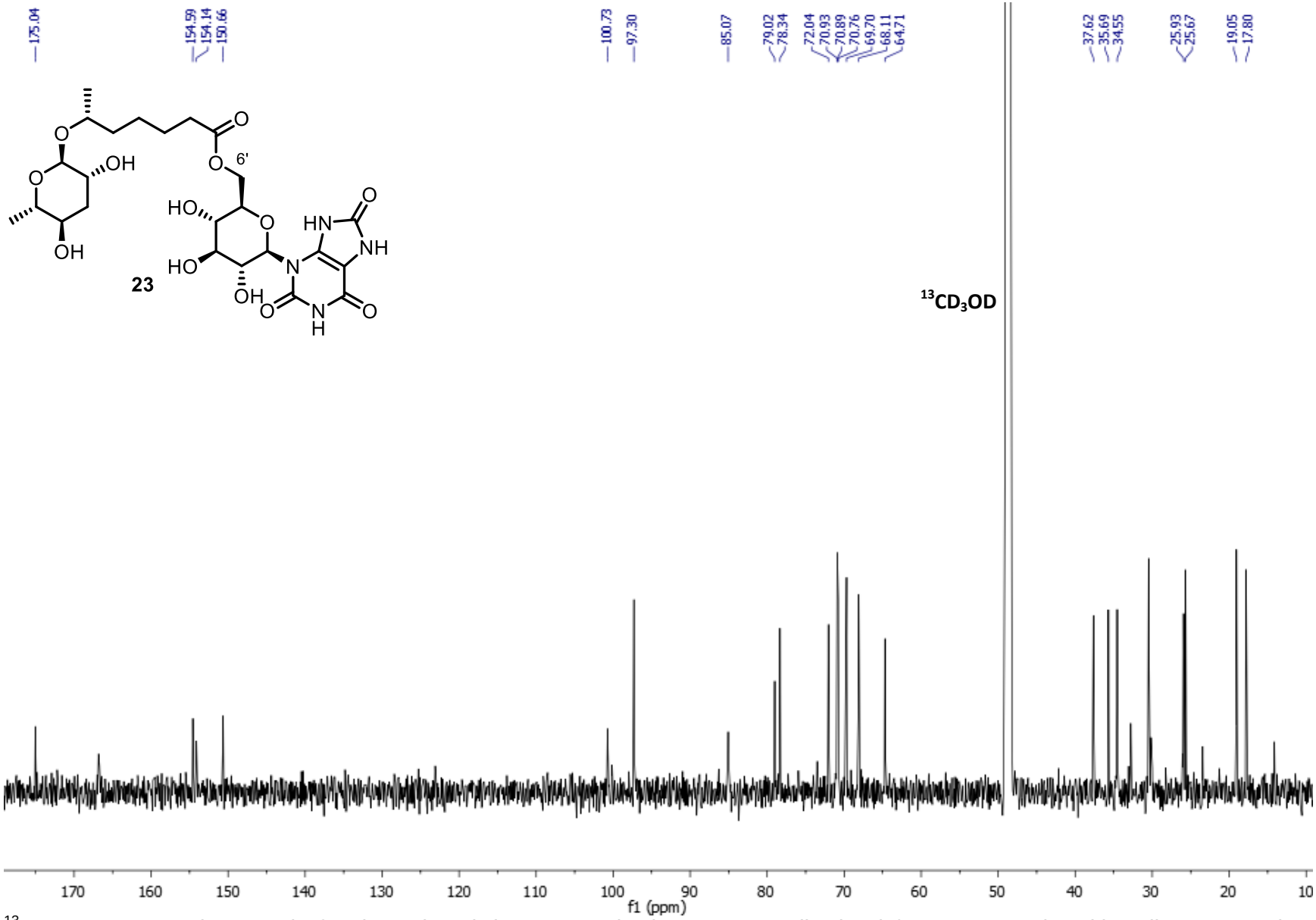
^{1}H NMR spectrum (800 MHz) of **19** and **20** (2:1) in methanol-d_4.



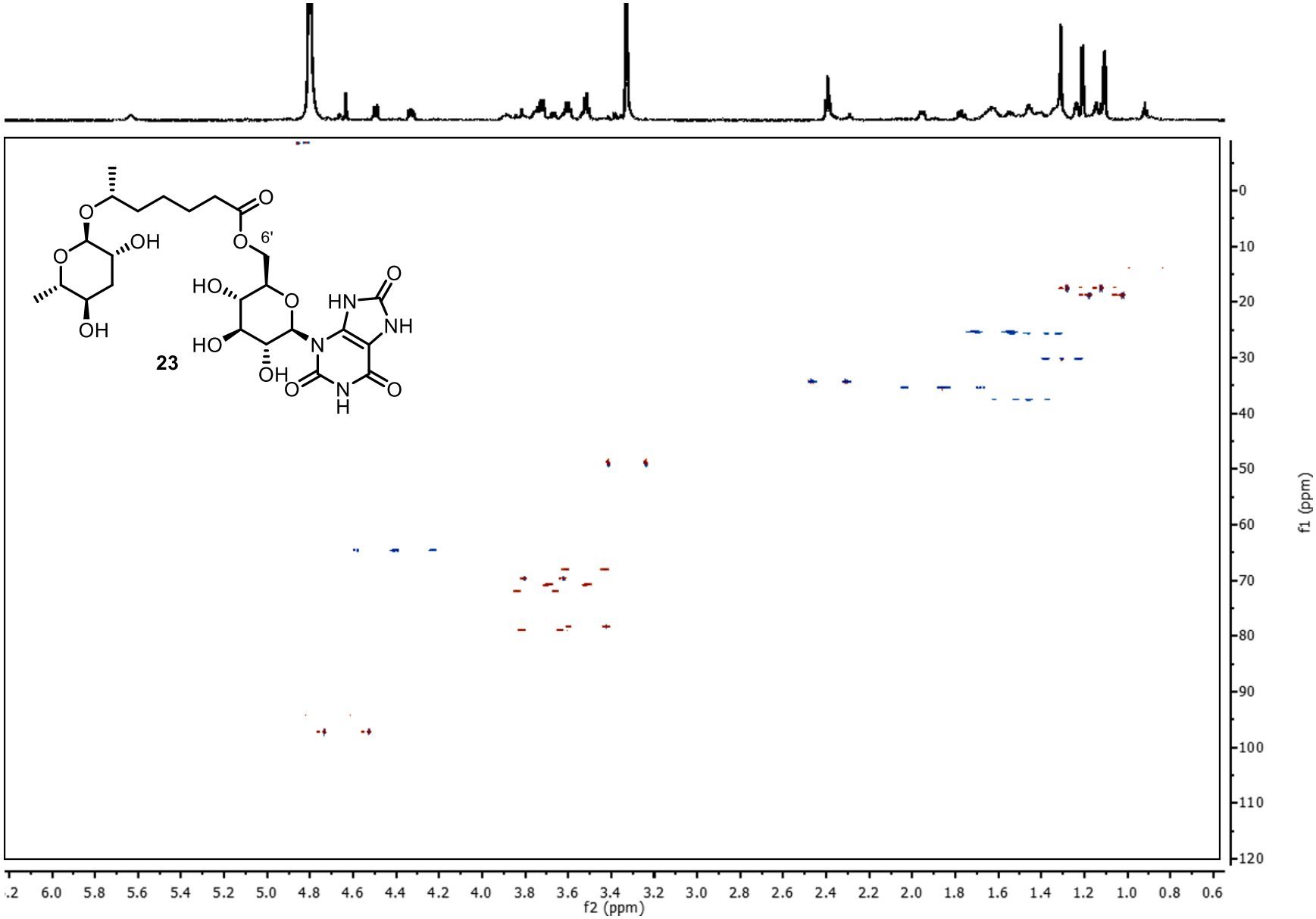
dqfCOSY spectrum (600 MHz) of a mixture of **19** and **20** (2:1) in methanol-d_4.



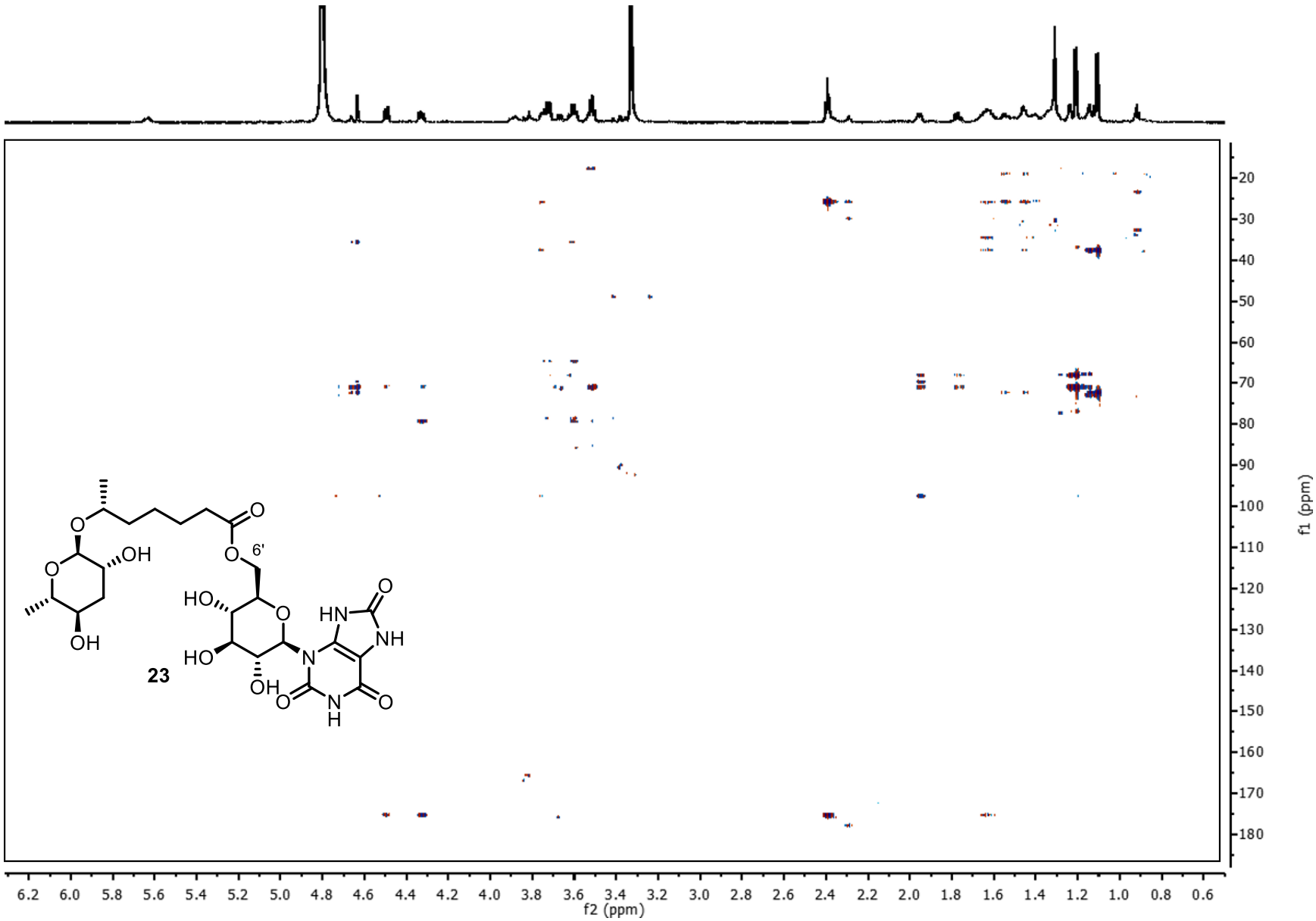
[1]H NMR spectrum (800 MHz) of **23** in methanol-d_4. This sample contains a small amount of **21** as an impurity marked with *.



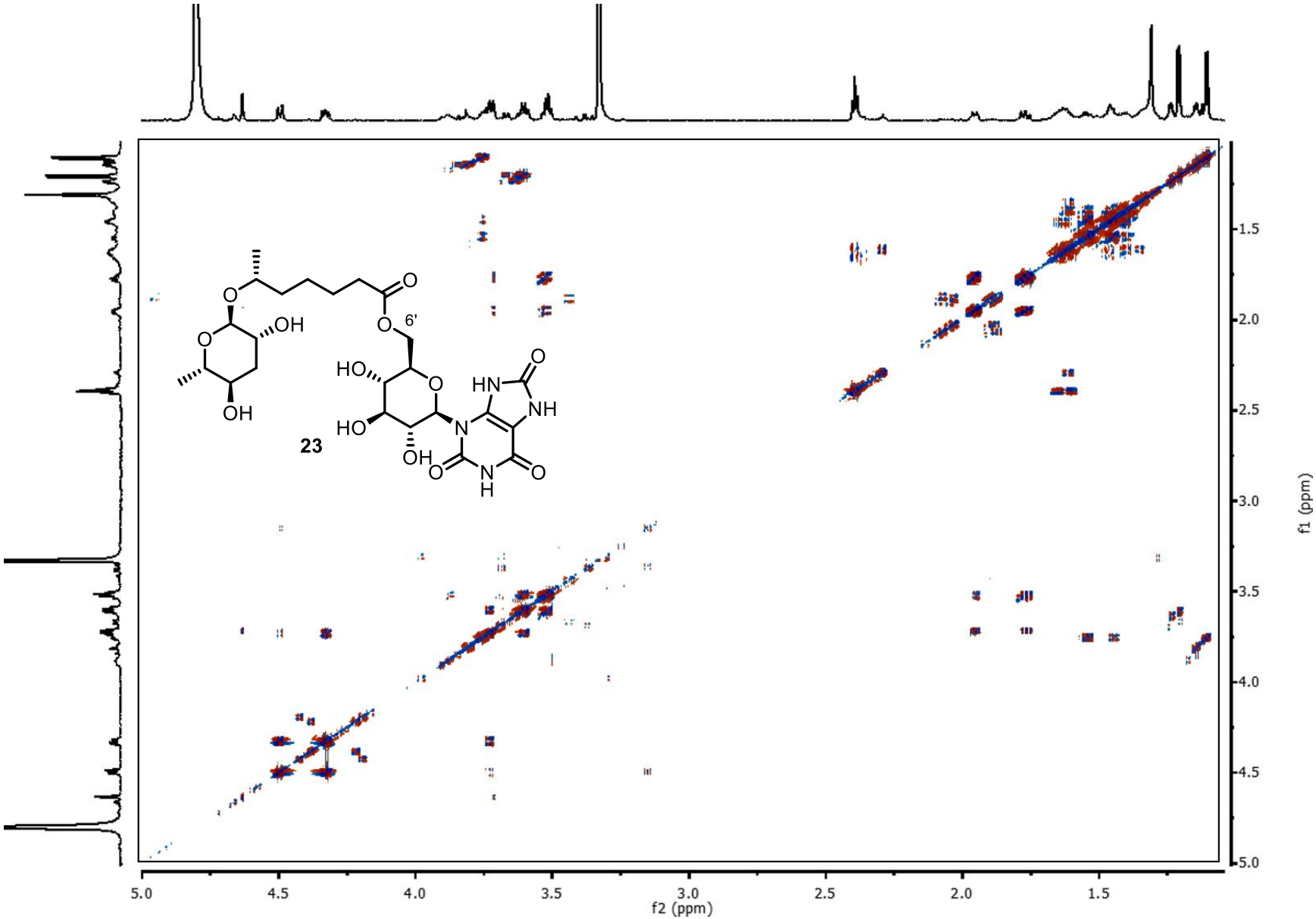
^{13}C NMR spectrum (201 MHz) of **23** in methanol-d_4. Processed using strong apodization (gf = 10, MNOVA) and baseline corrected using the Whittaker Smoother (MNOVA).



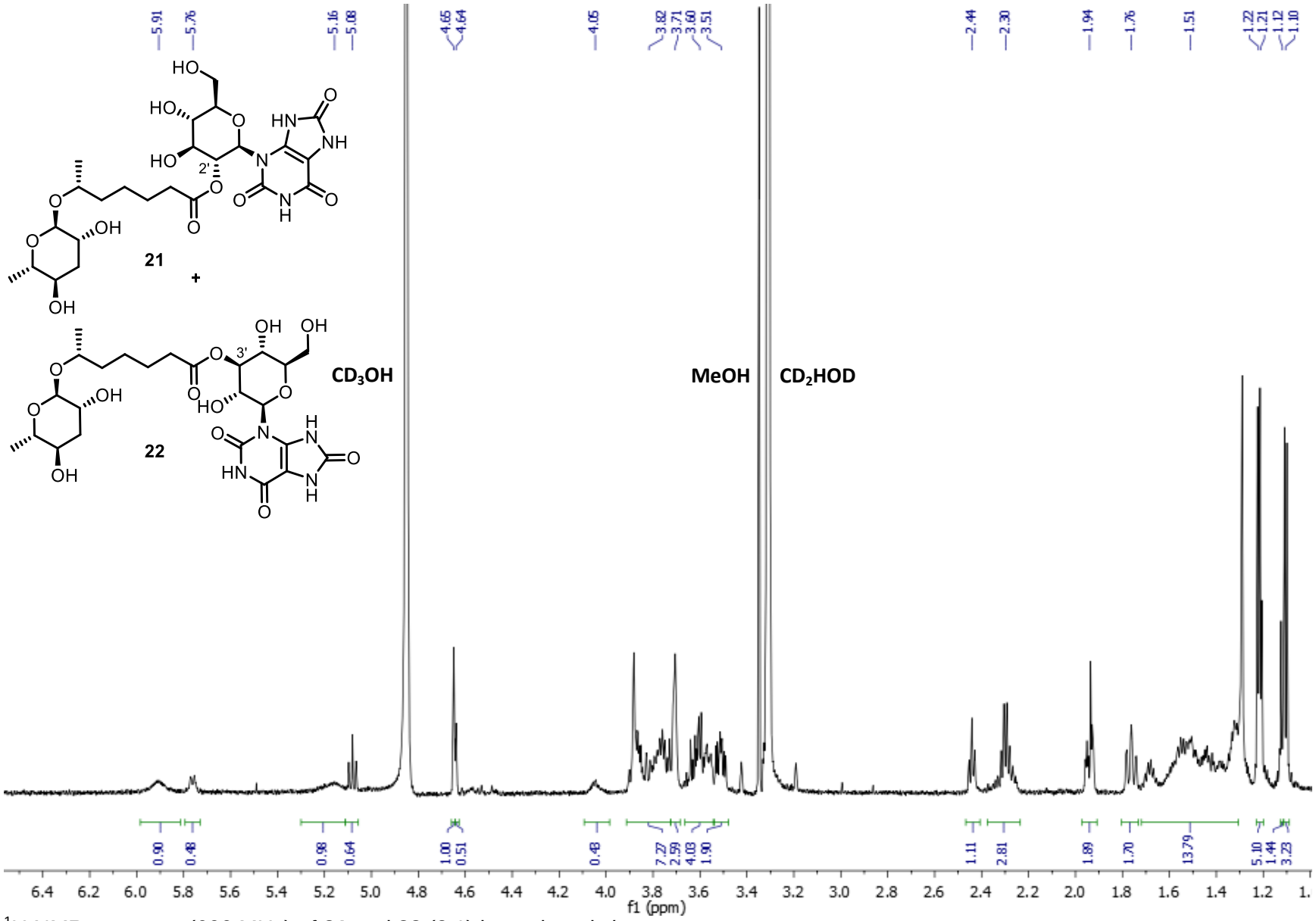
HSQC spectrum (800 MHz) of **23** in methanol-d_4.



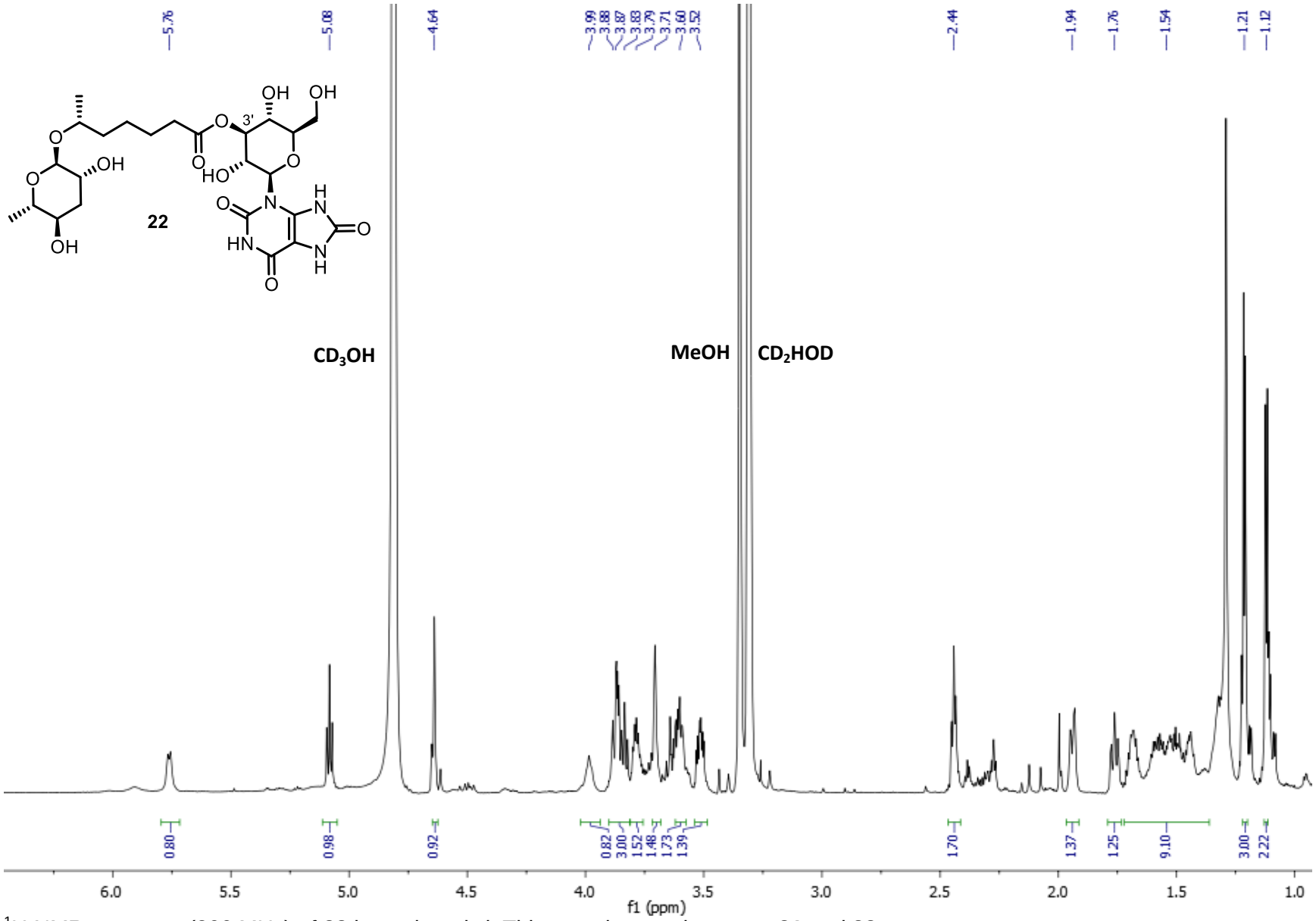
HMBC spectrum (800 MHz) of **23** in methanol-d_4.



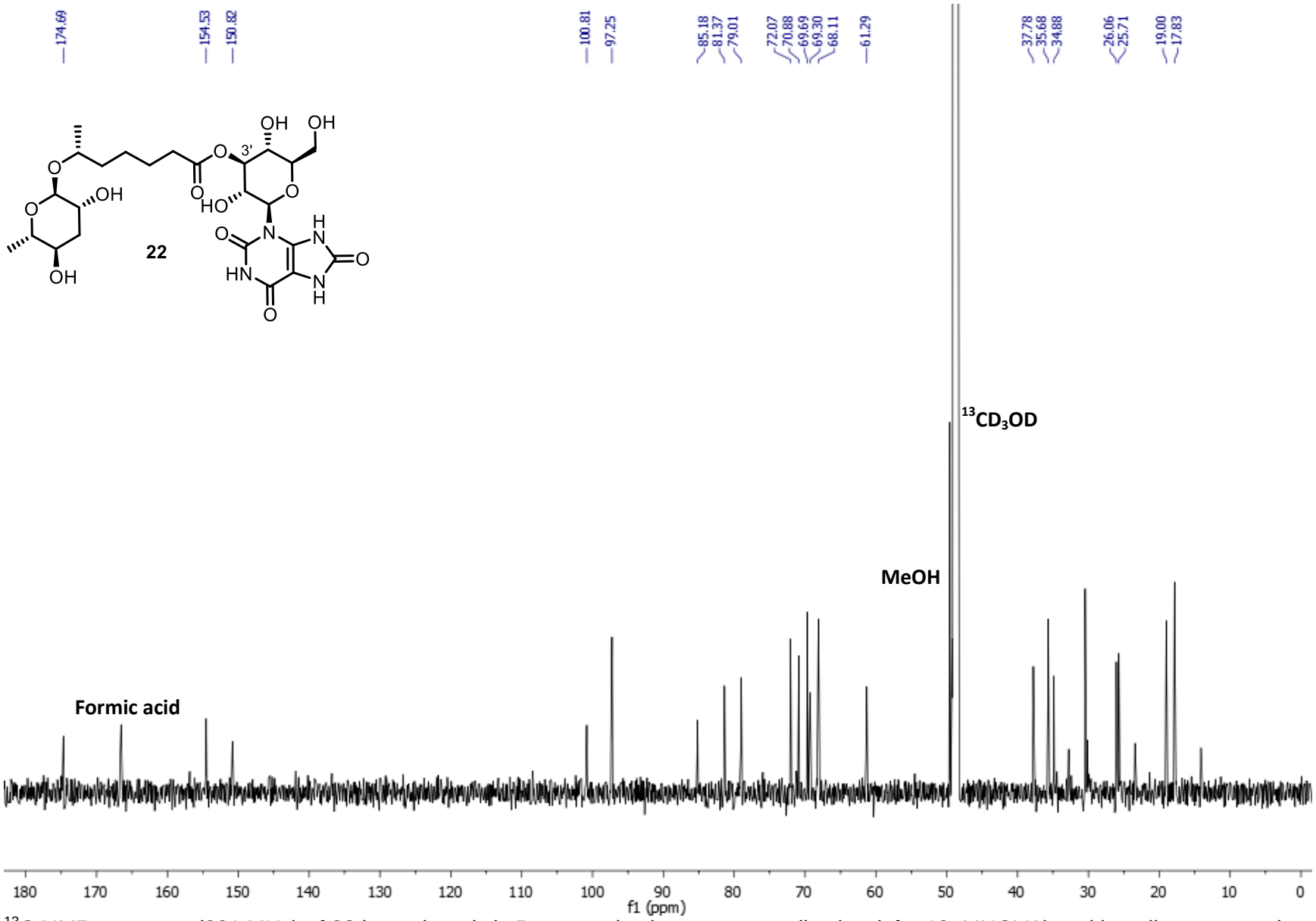
dqfCOSY spectrum (800 MHz) of **23** in methanol-d_4.



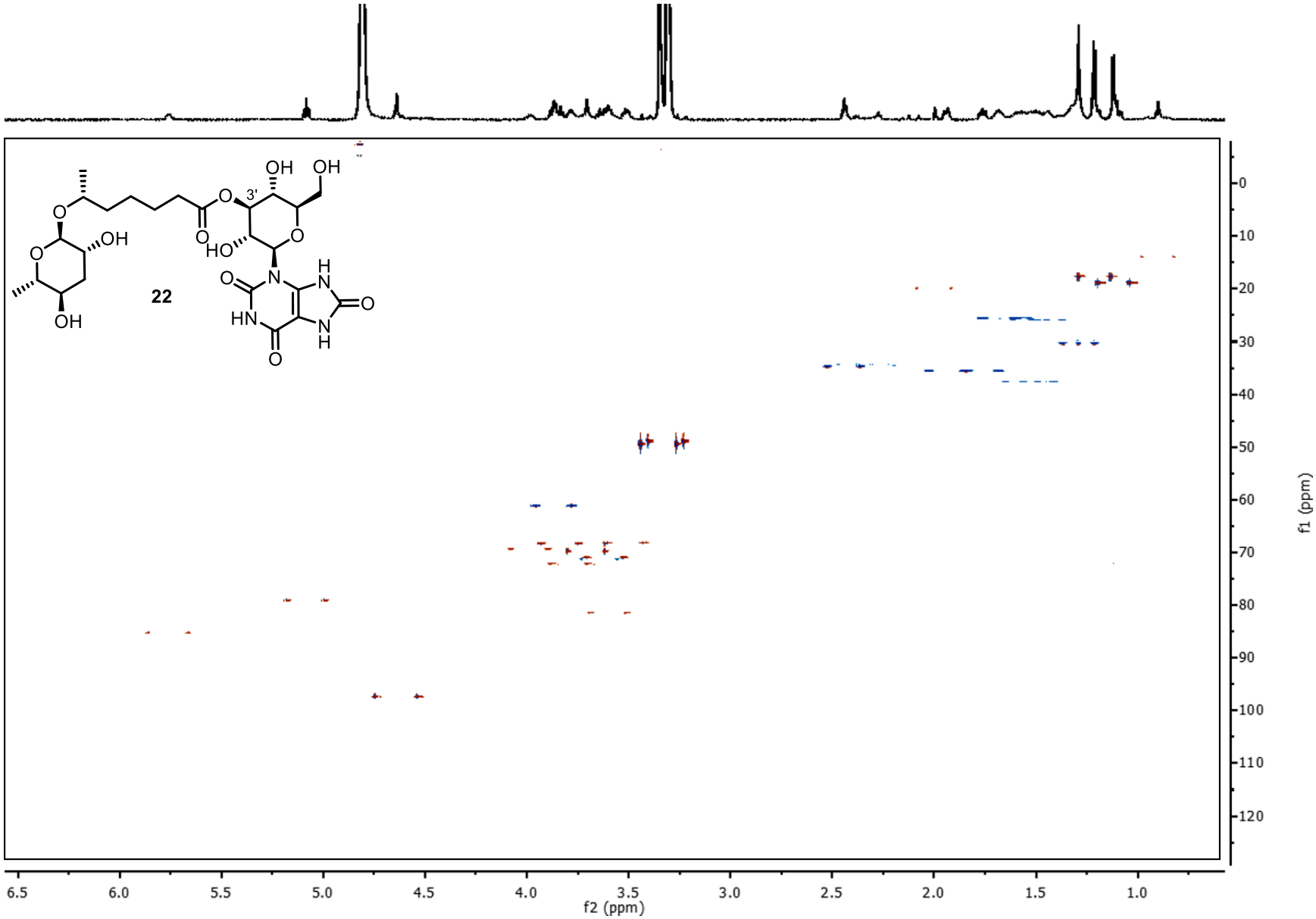
^{1}H NMR spectrum (600 MHz) of **21** and **22** (2:1) in methanol-d_4.



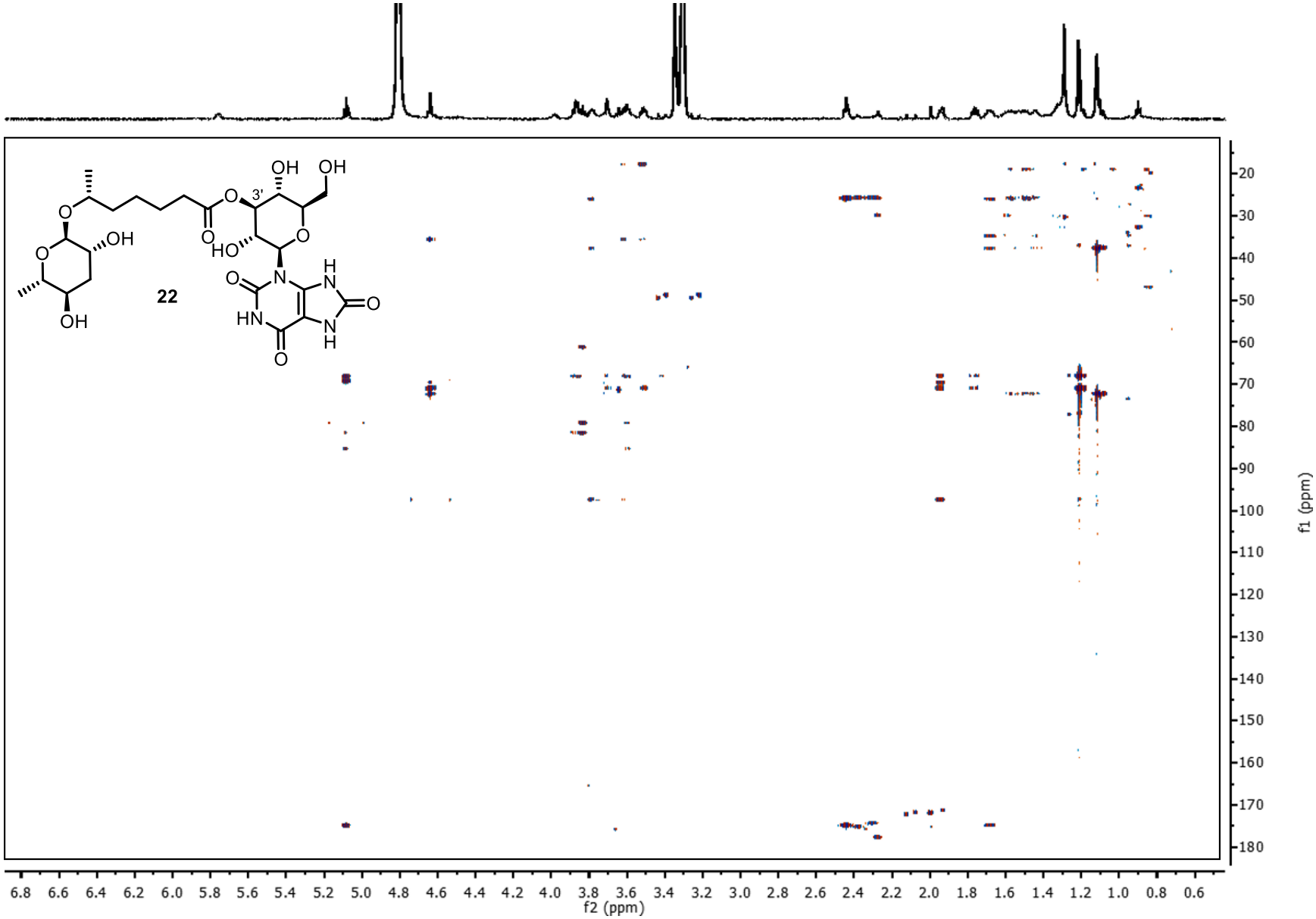
^{1}H NMR spectrum (800 MHz) of **22** in methanol-d_4. This sample contains some **21** and **23**.



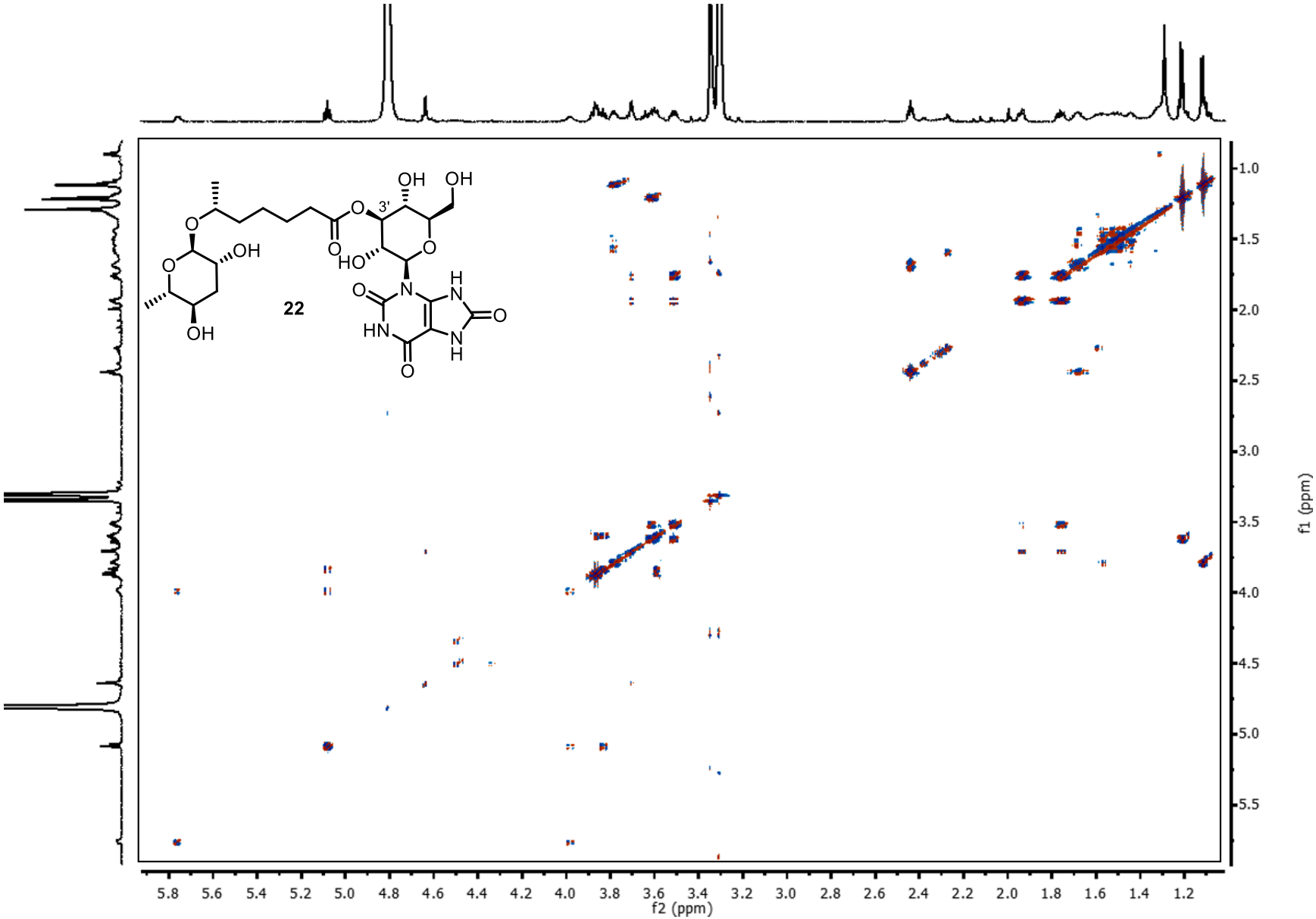
^{13}C NMR spectrum (201 MHz) of **22** in methanol-d_4. Processed using strong apodization (gf = 10, MNOVA) and baseline corrected using the Whittaker Smoother (MNOVA).



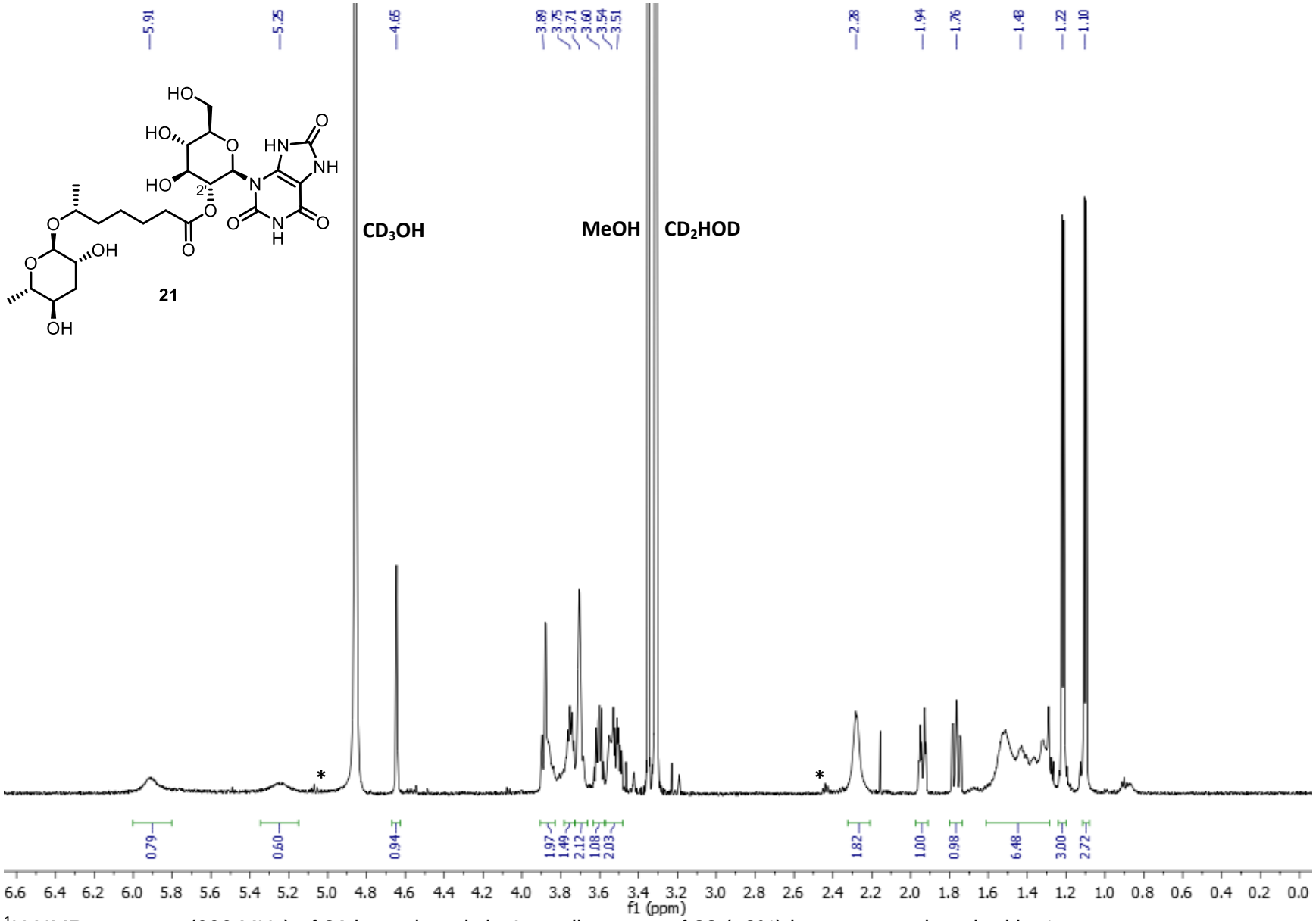
HSQC spectrum (800 MHz) of **22** in methanol-d_4.



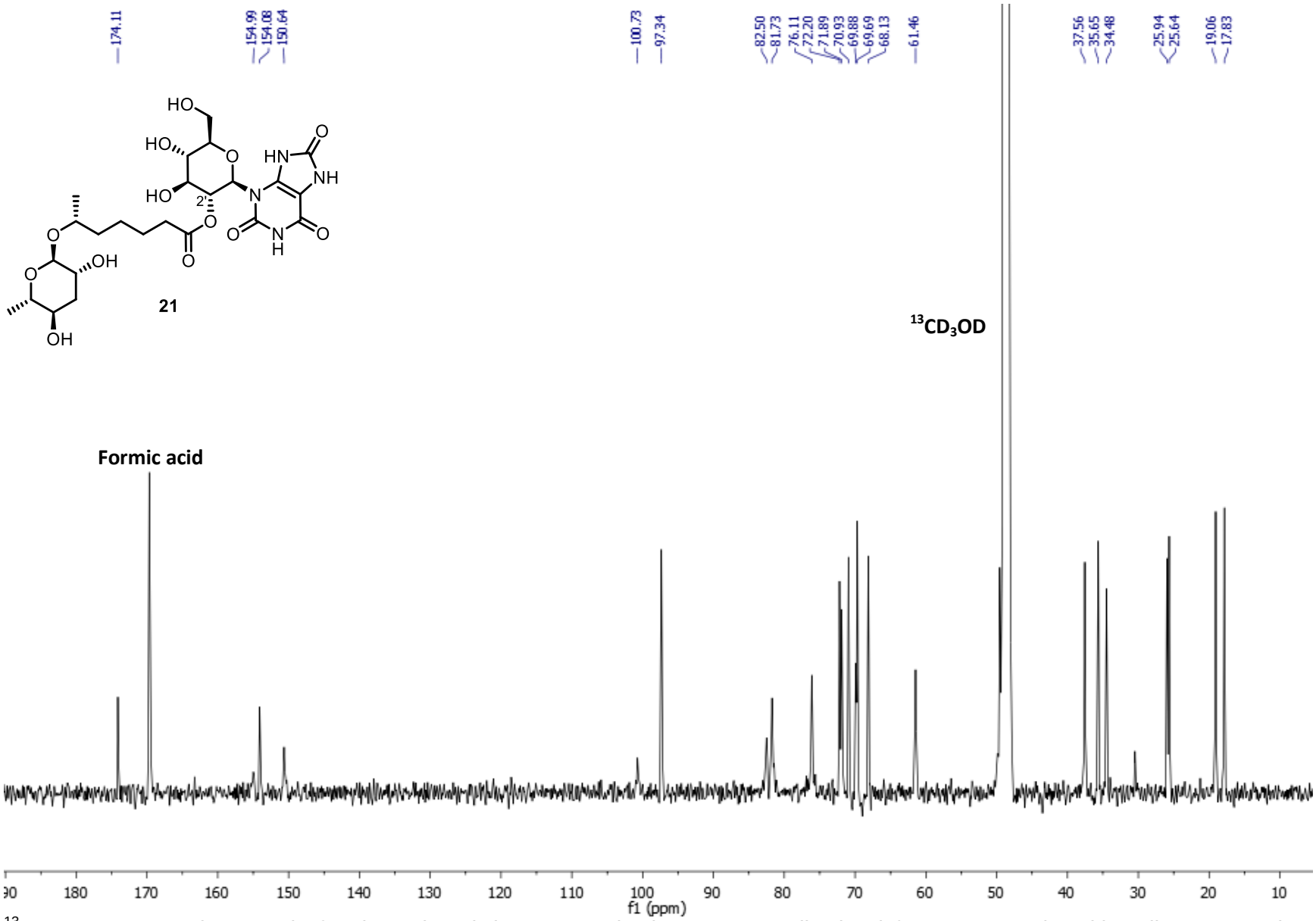
HMBC spectrum (800 MHz) of **22** in methanol-d_4.



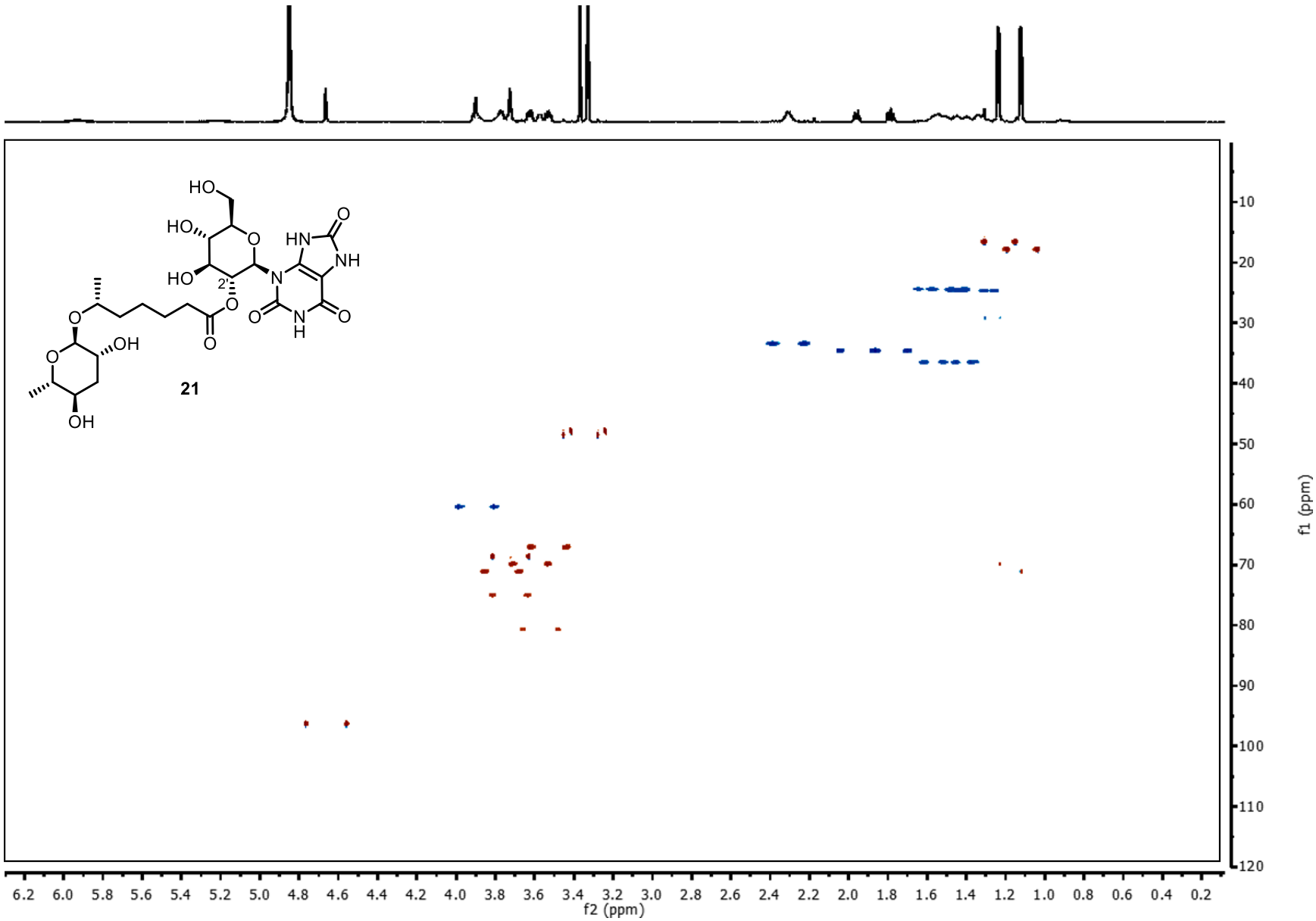
dqfCOSY spectrum (800 MHz) of **22** in methanol-d_4.



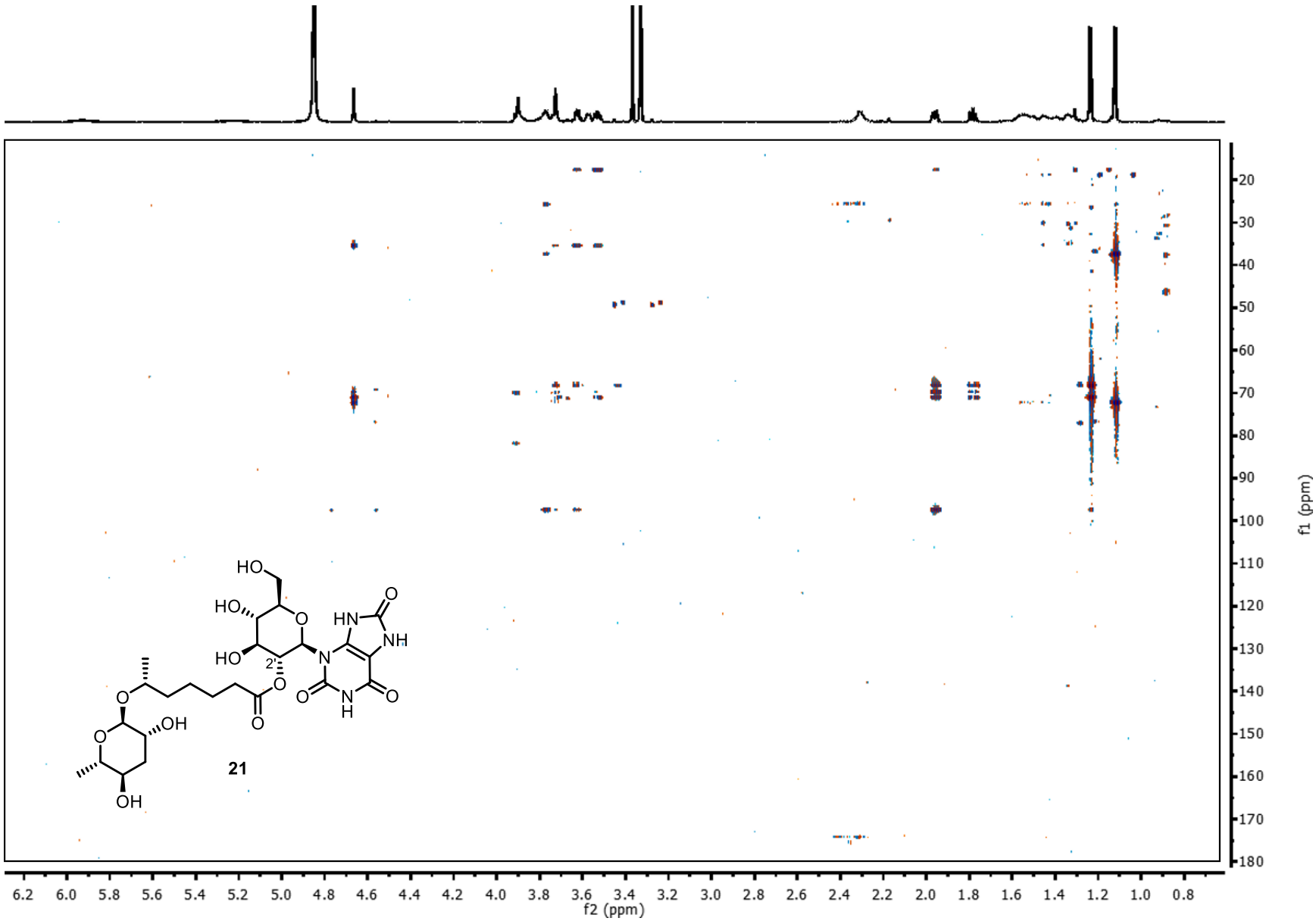
[1]H NMR spectrum (600 MHz) of **21** in methanol-d_4. A small amount of **22** (~2%) is present and marked by *.



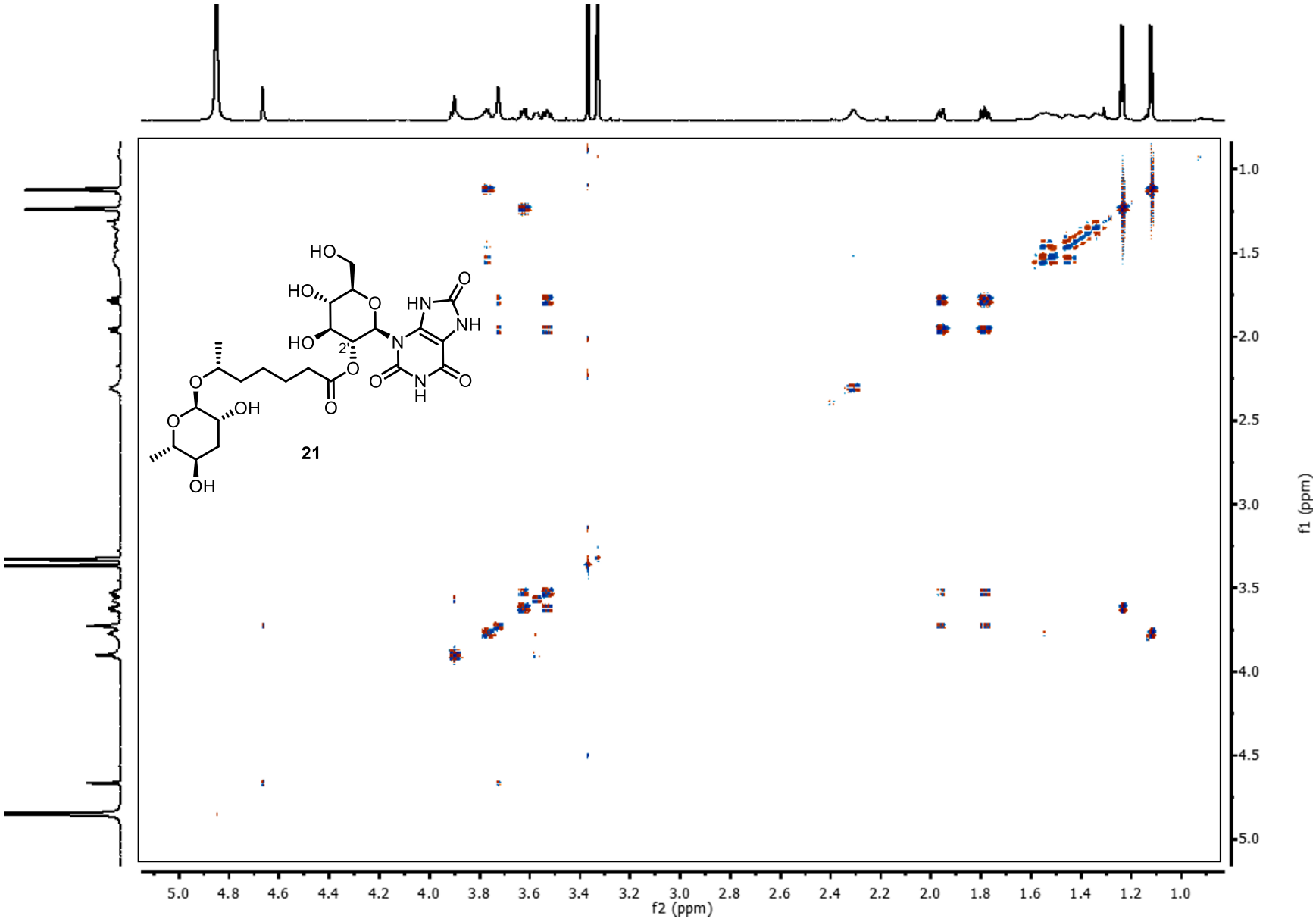
^{13}C NMR spectrum (201 MHz) of **21** in methanol-d_4. Processed using strong apodization (gf = 22, MNOVA) and baseline corrected using the Whittaker Smoother (MNOVA).



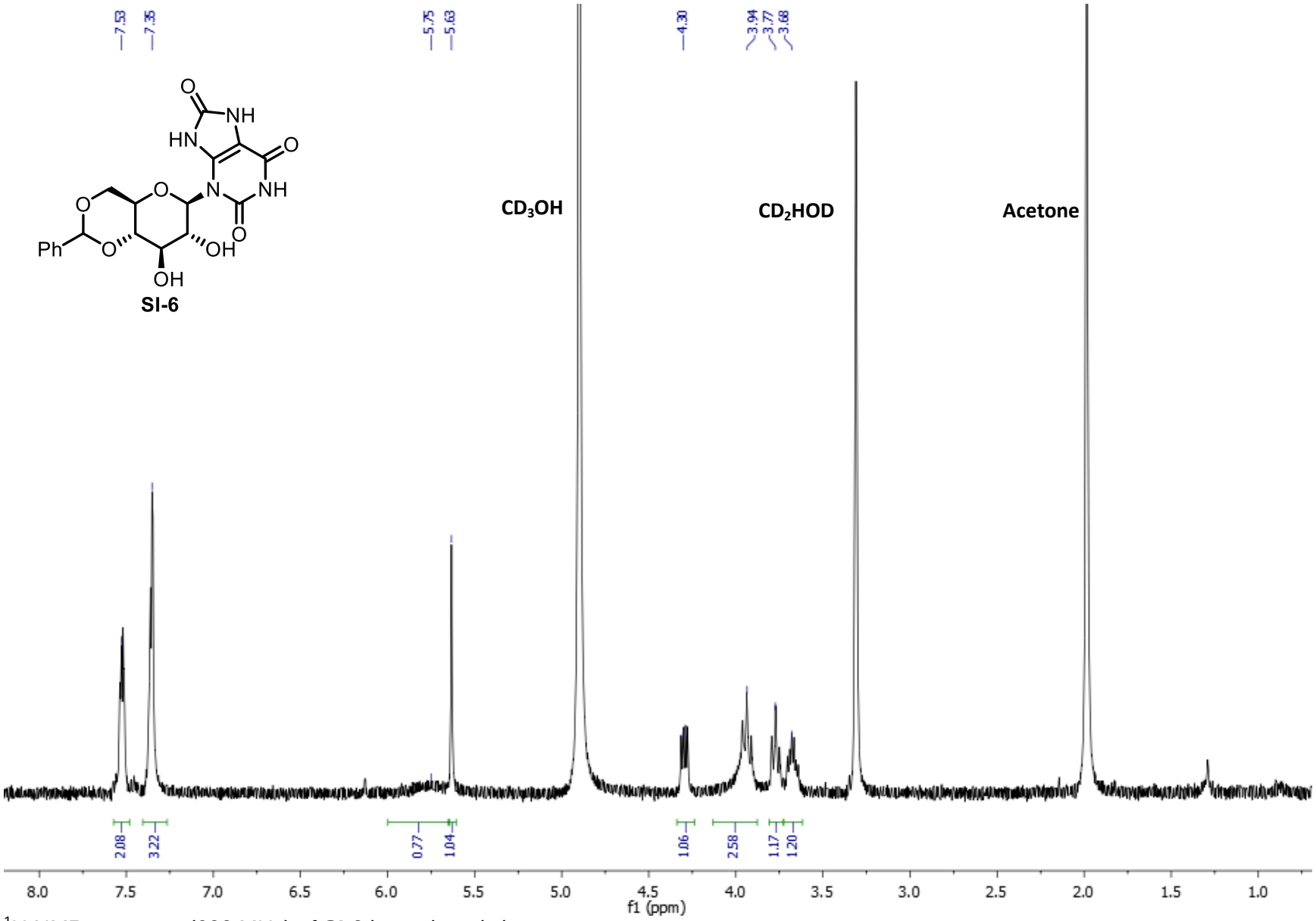
HSQC spectrum (800 MHz) of **21** in methanol-d_4.



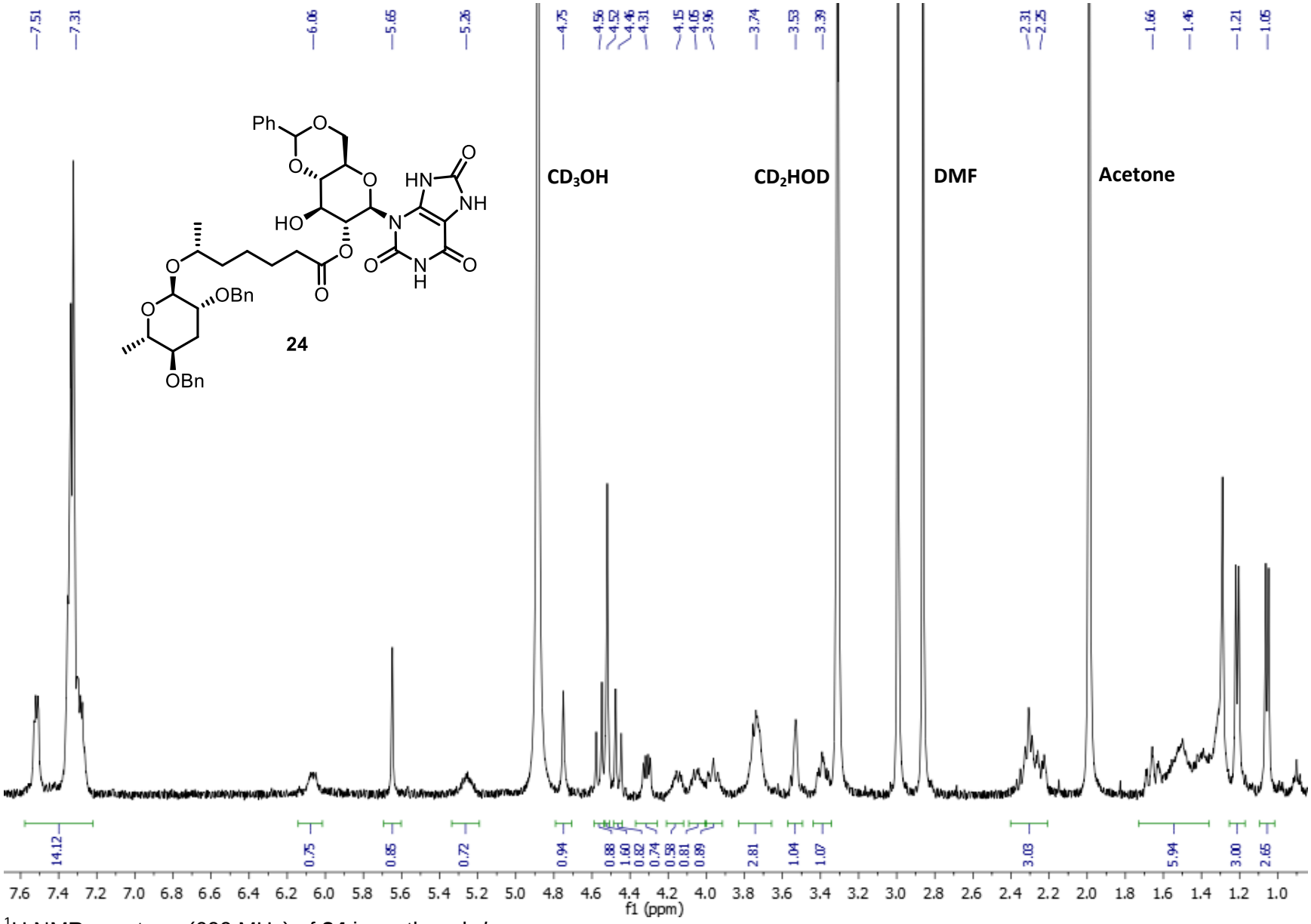
HMBC spectrum (800 MHz) of **21** in methanol-d_4.



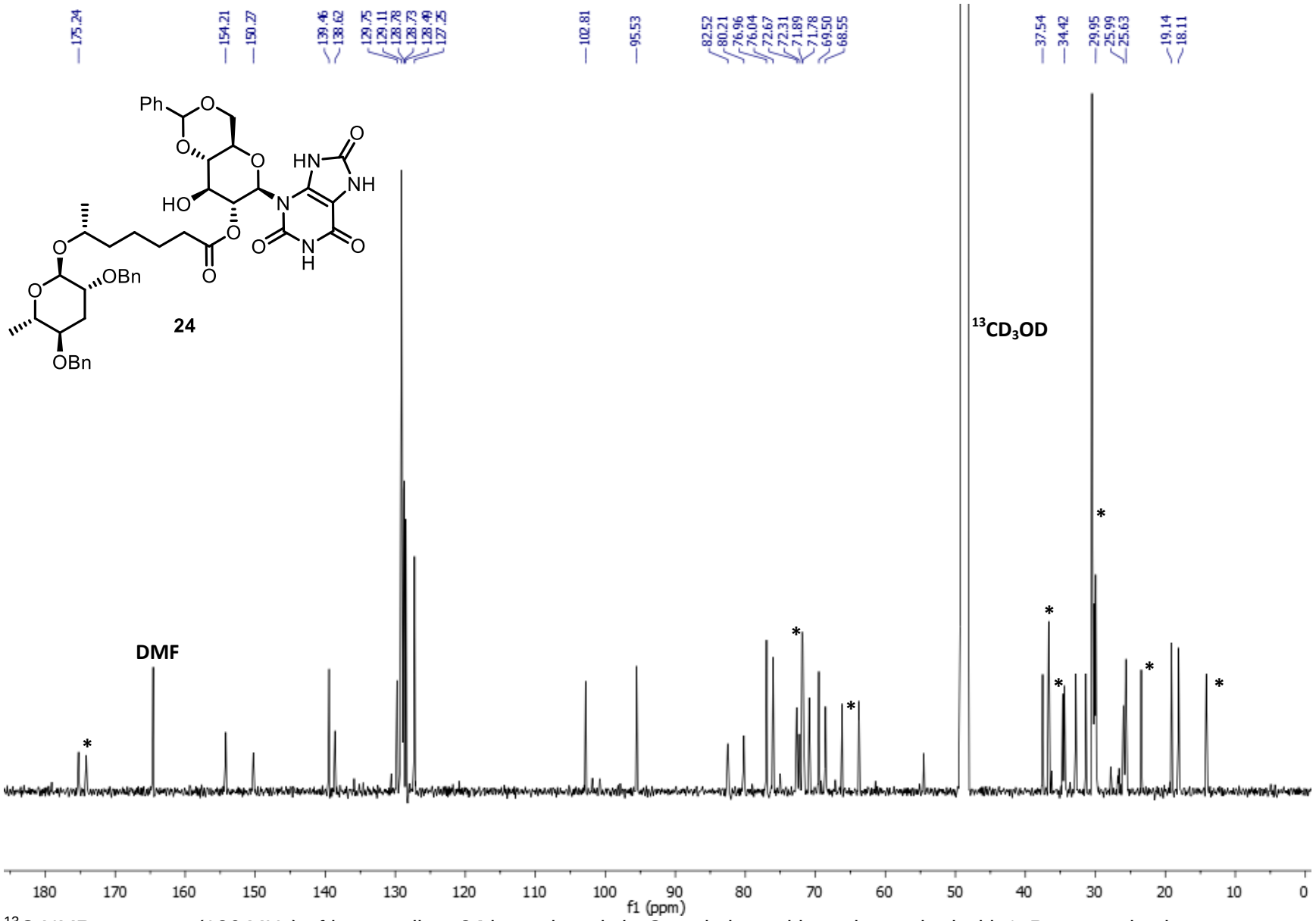
dqfCOSY spectrum (800 MHz) of **21** in methanol-d_4.



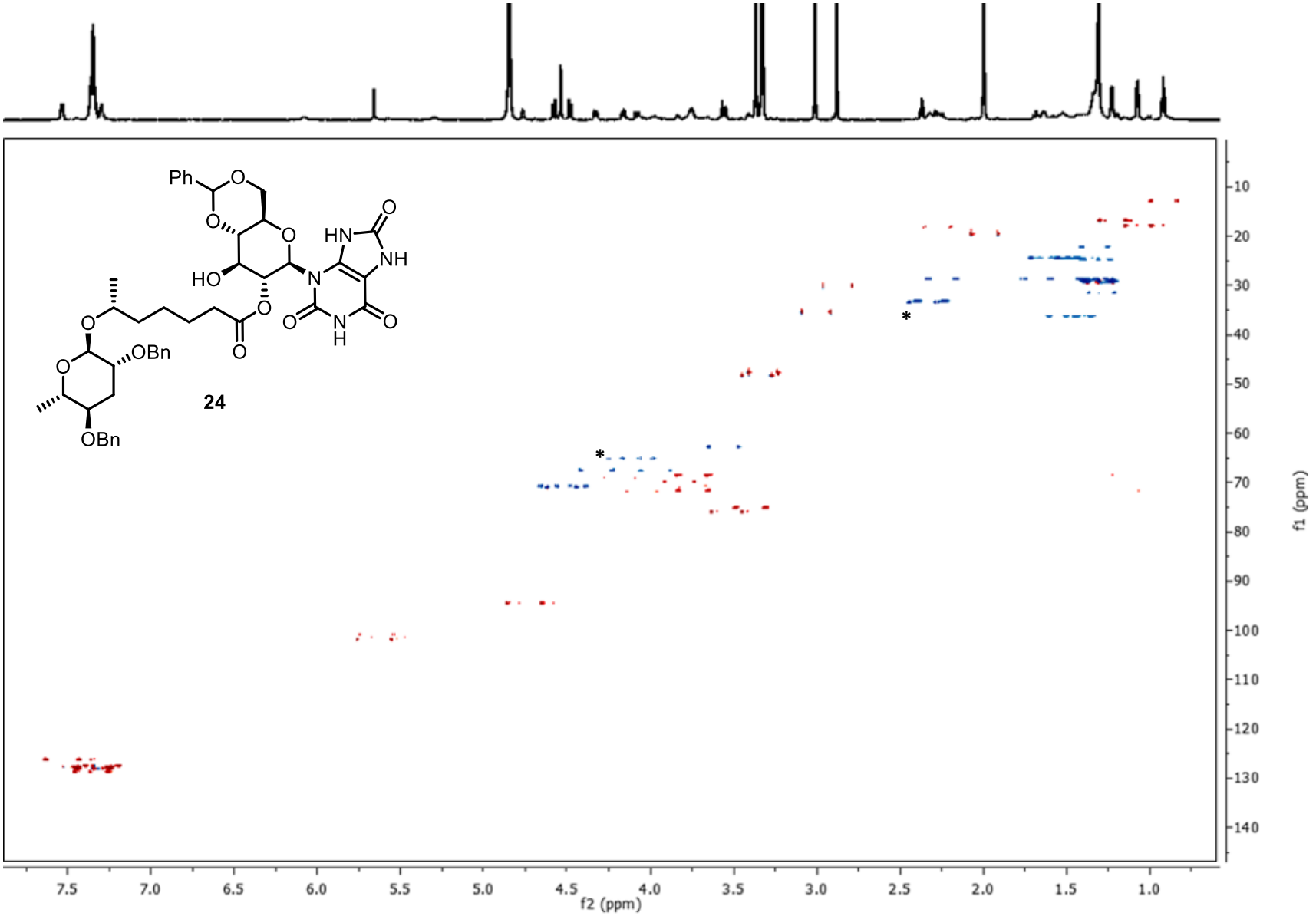
^{1}H NMR spectrum (600 MHz) of **SI-6** in methanol-d_4.



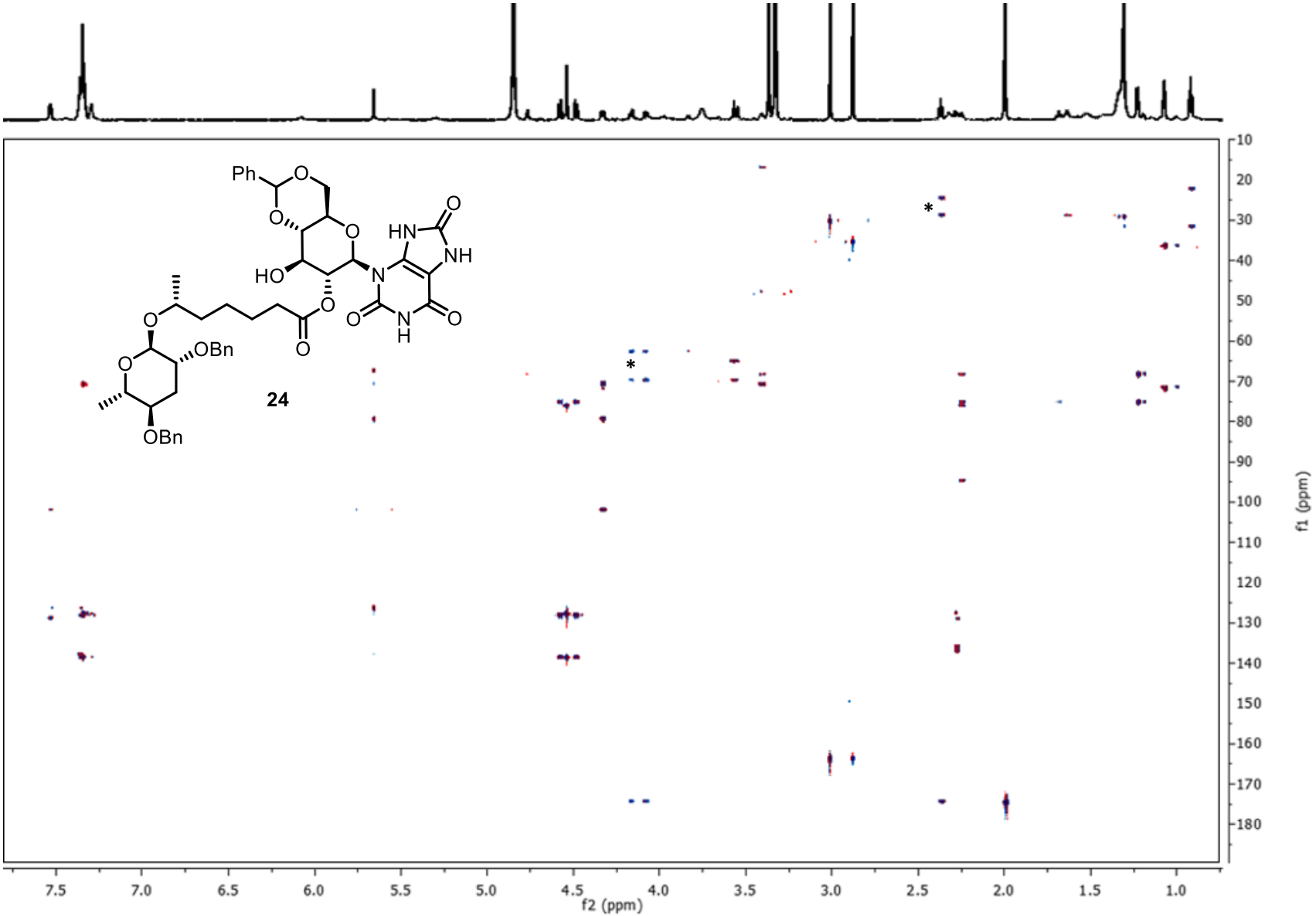
^{1}H NMR spectrum (600 MHz) of **24** in methanol-d_4.



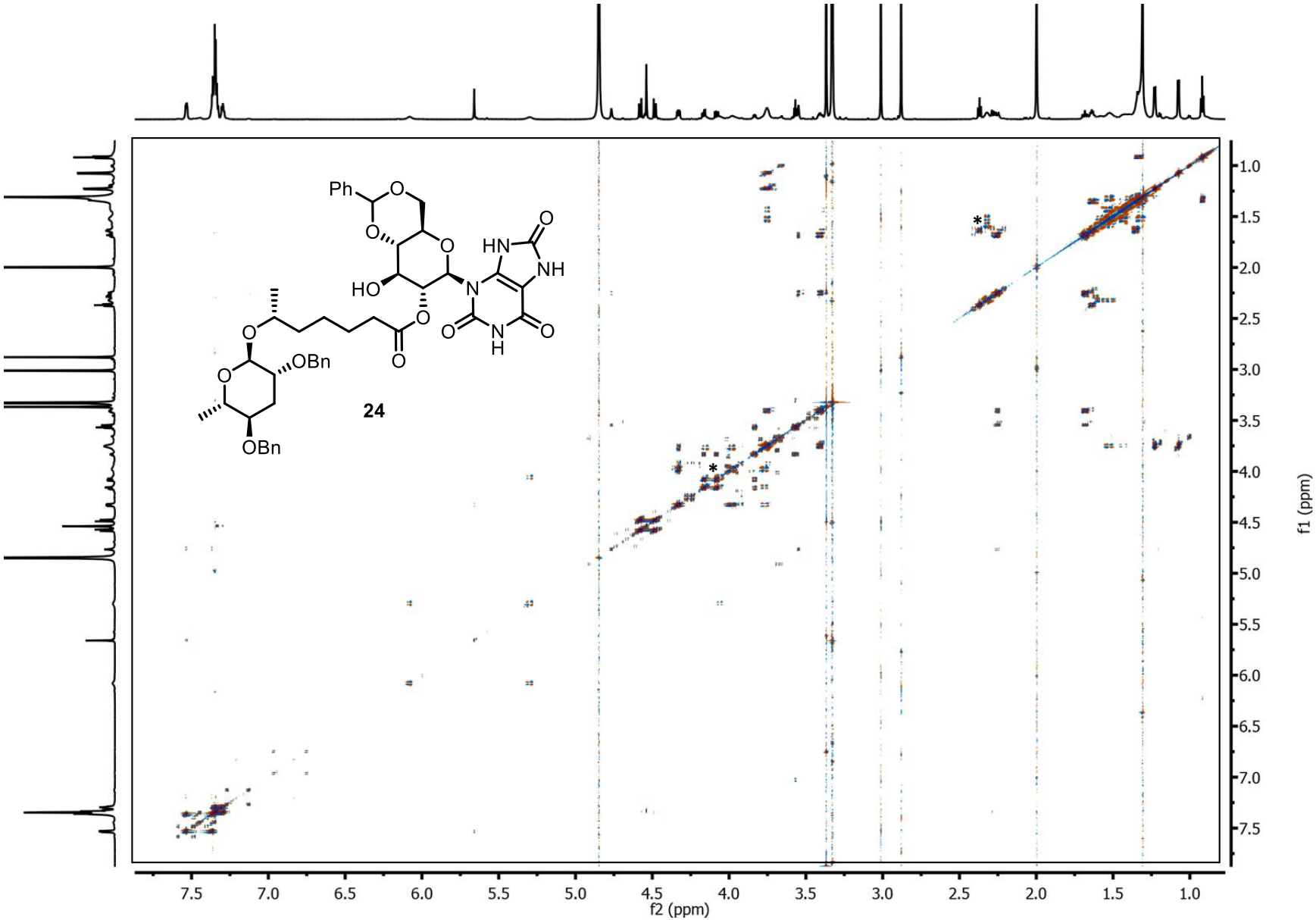
^{13}C NMR spectrum (126 MHz) of intermediate **24** in methanol-d_4. *O*-acyl glycerol impurity marked with *. Processed using strong apodization (gf = 10, MNOVA) and baseline corrected using the Whittaker Smoother (MNOVA).



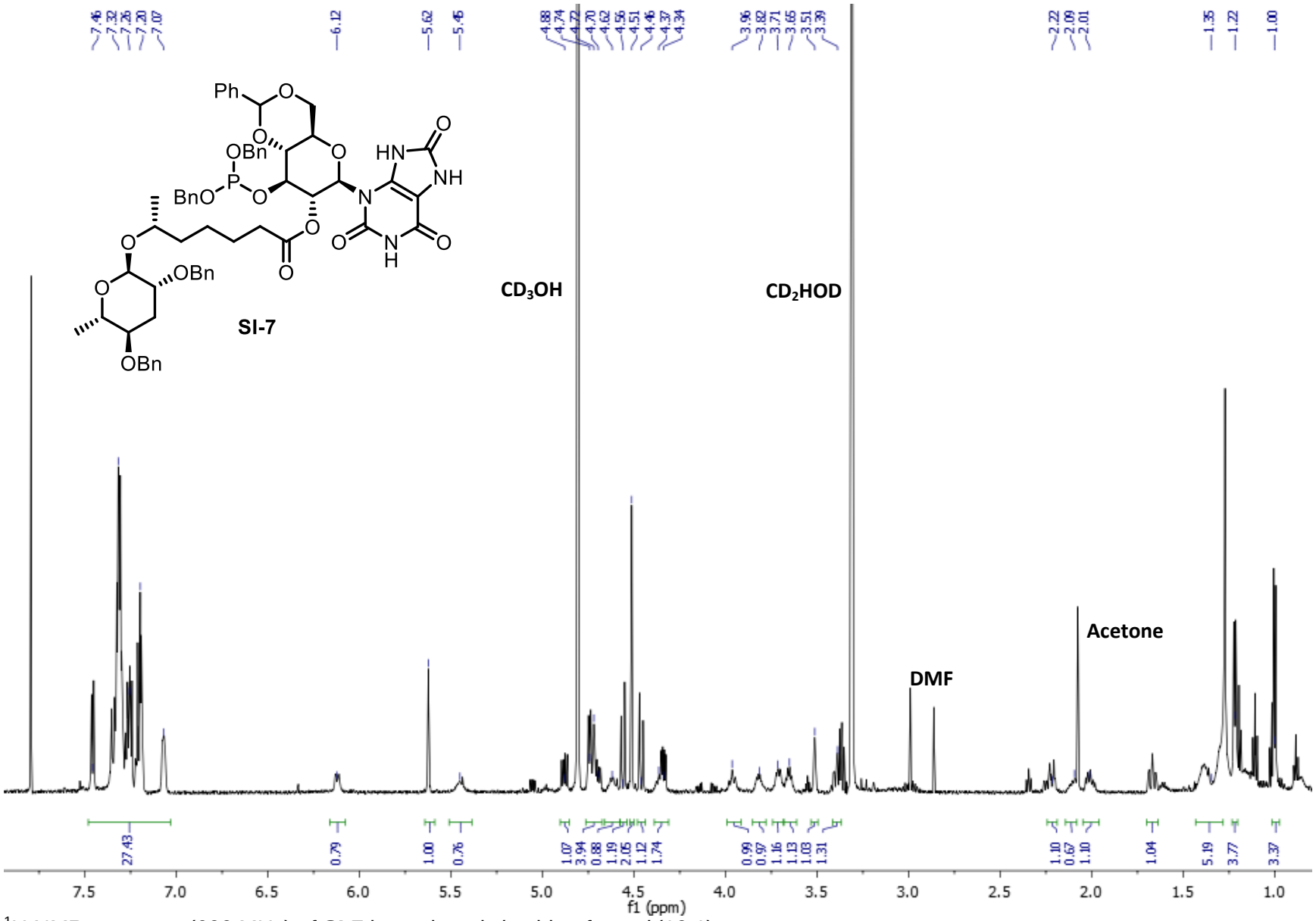
HSQC spectrum (800 MHz) of **24** in methanol-d_4. *O*-acyl glycerol impurity marked with *.



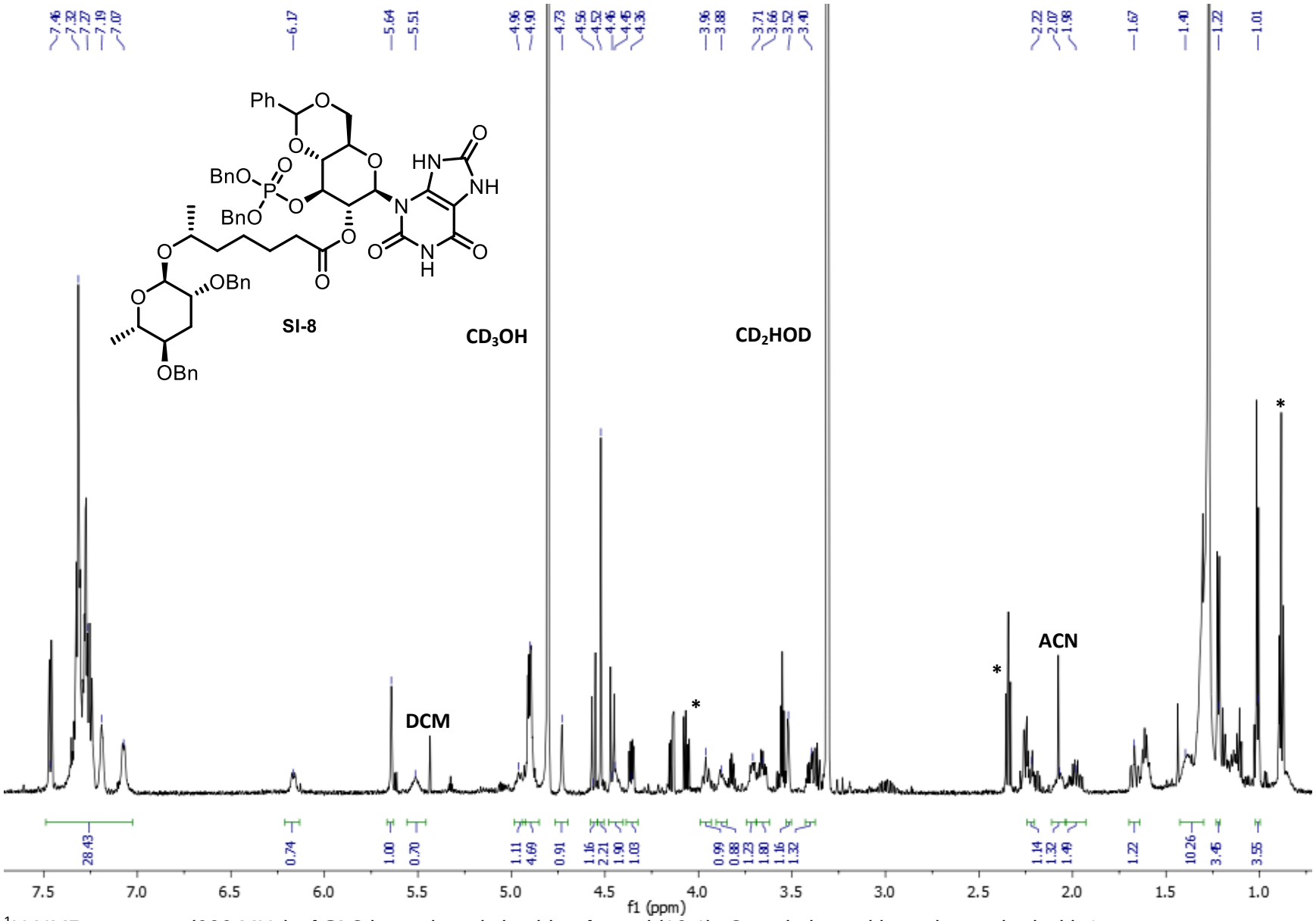
HMBC spectrum (800 MHz) of **24** in methanol-d_4. *O*-acyl glycerol impurity marked with *.



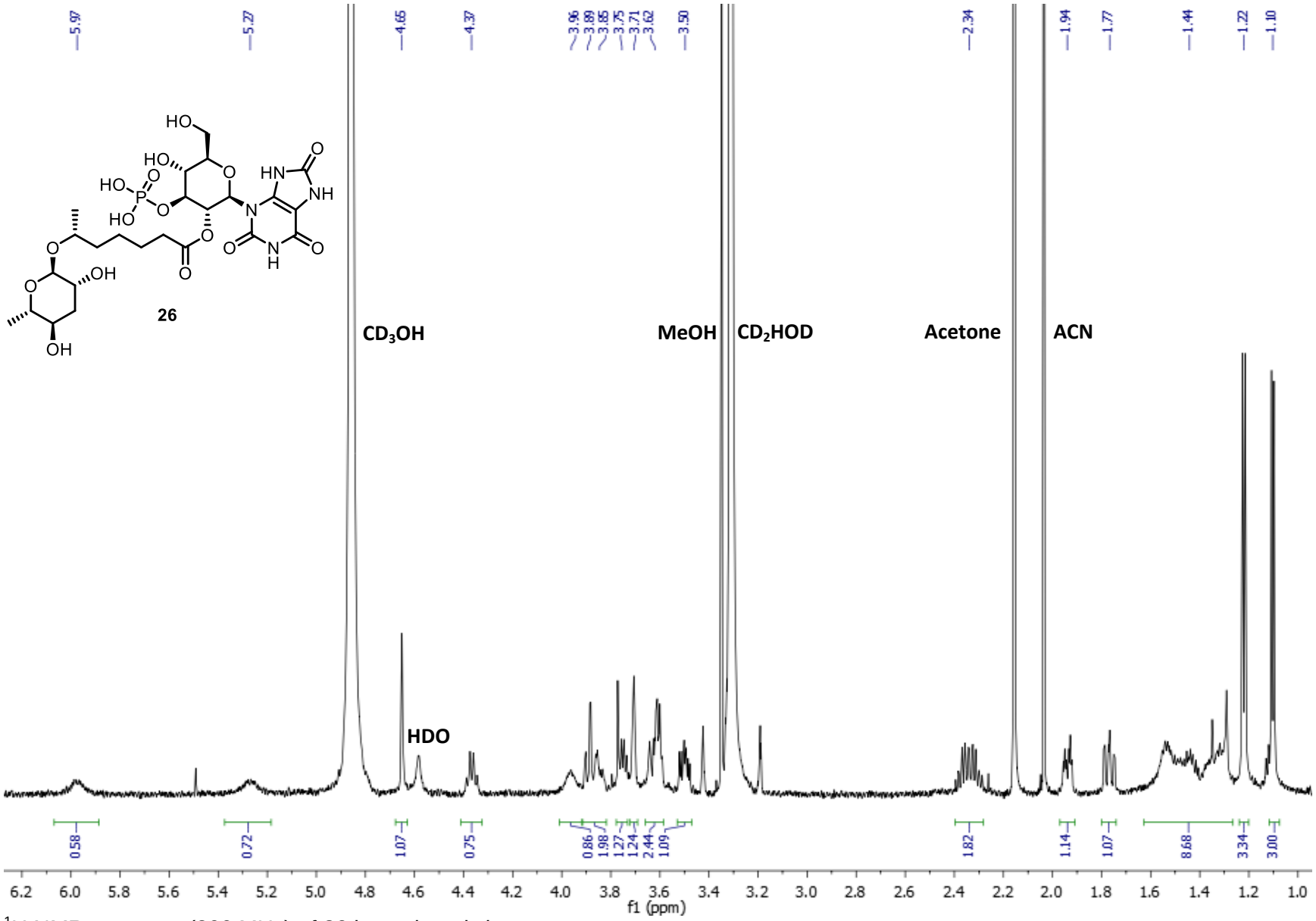
dqfCOSY spectrum (800 MHz) of **24** in methanol-d_4. *O*-acyl glycerol impurity marked with *.



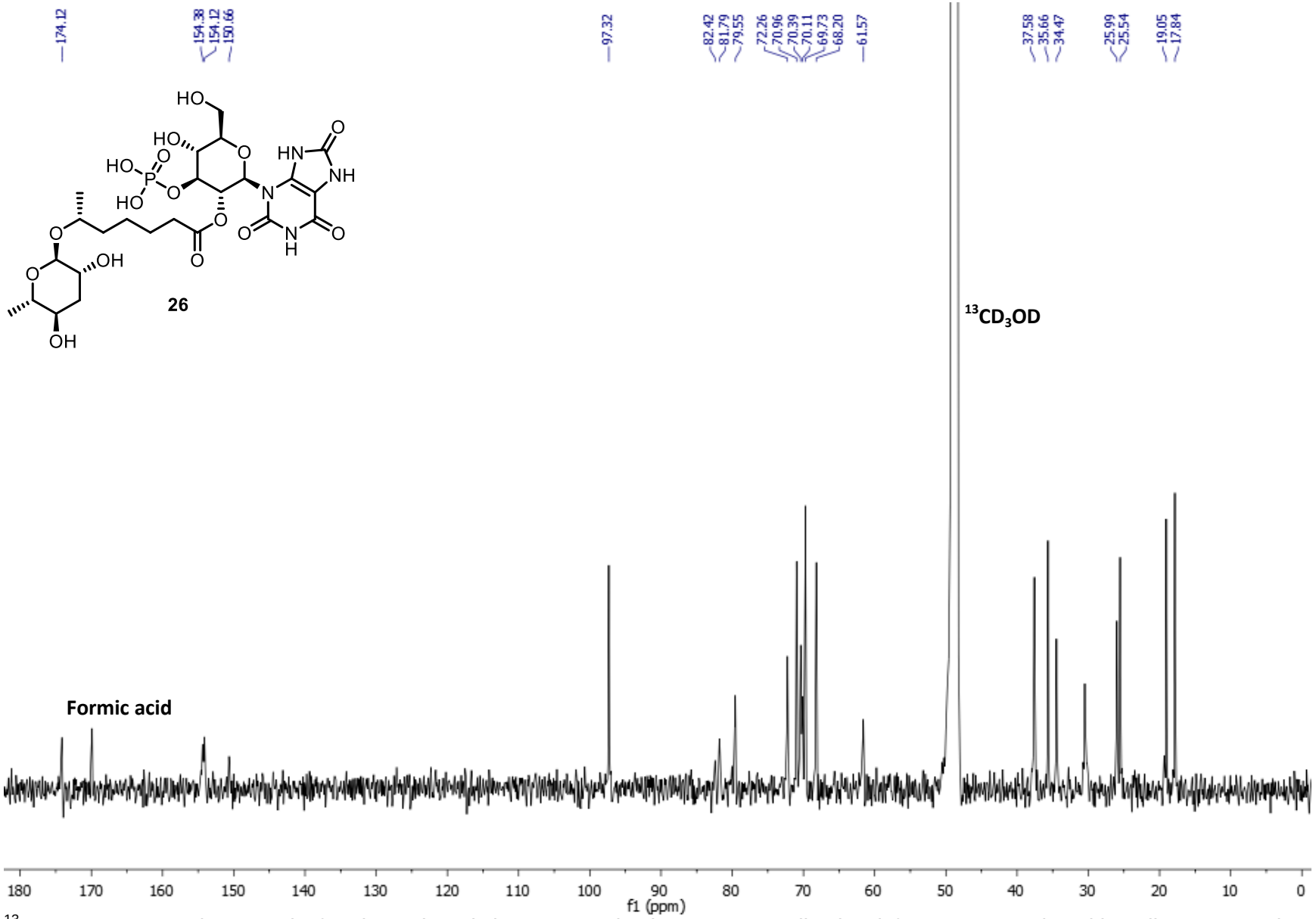
^{1}H NMR spectrum (600 MHz) of **SI-7** in methanol-d_4, chloroform-*d* (10:1).



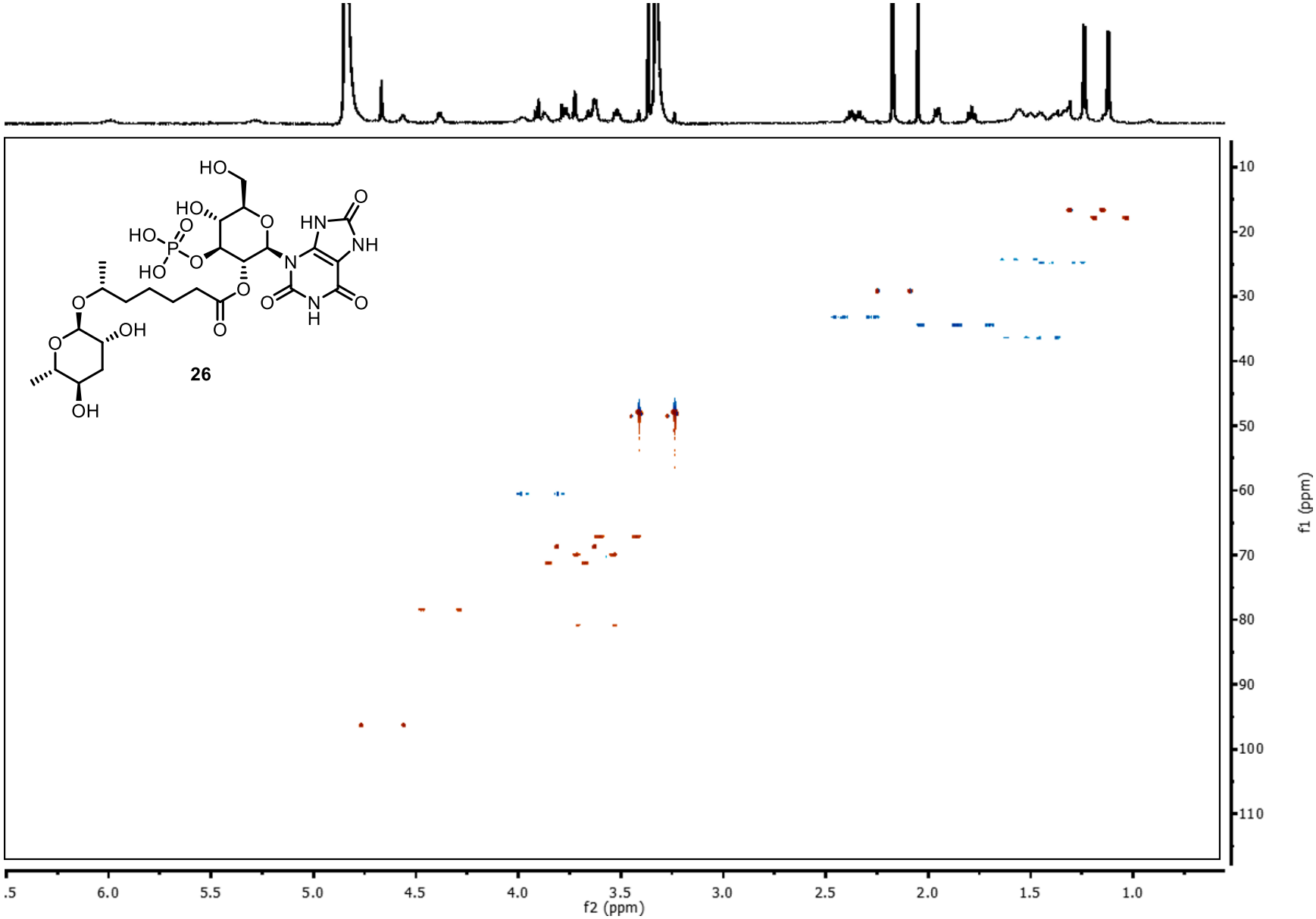
^{1}H NMR spectrum (600 MHz) of **SI-8** in methanol-d_4, chloroform-*d* (10:1). *O*-acyl glycerol impurity marked with *.



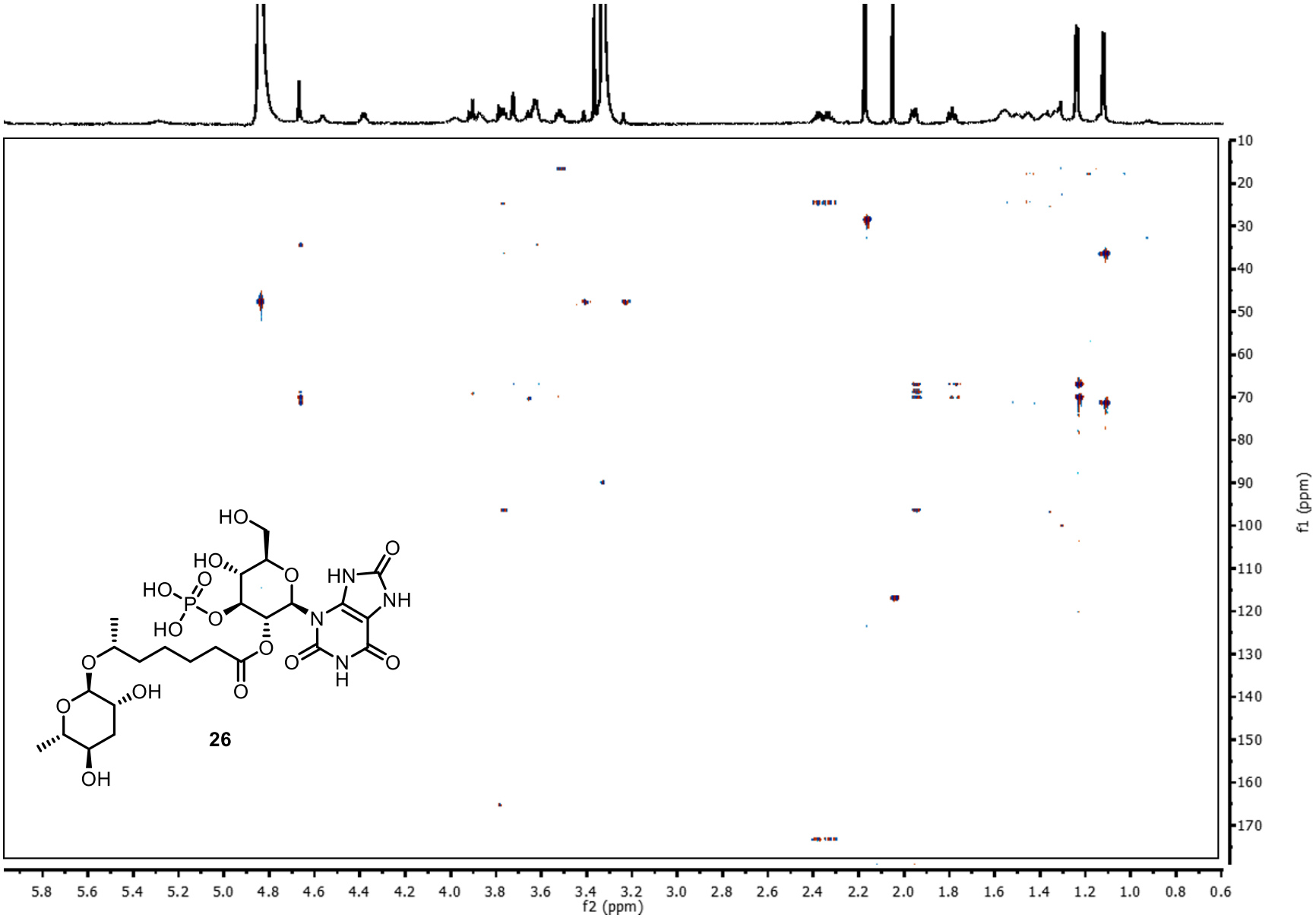
^{1}H NMR spectrum (800 MHz) of **26** in methanol-d_4.



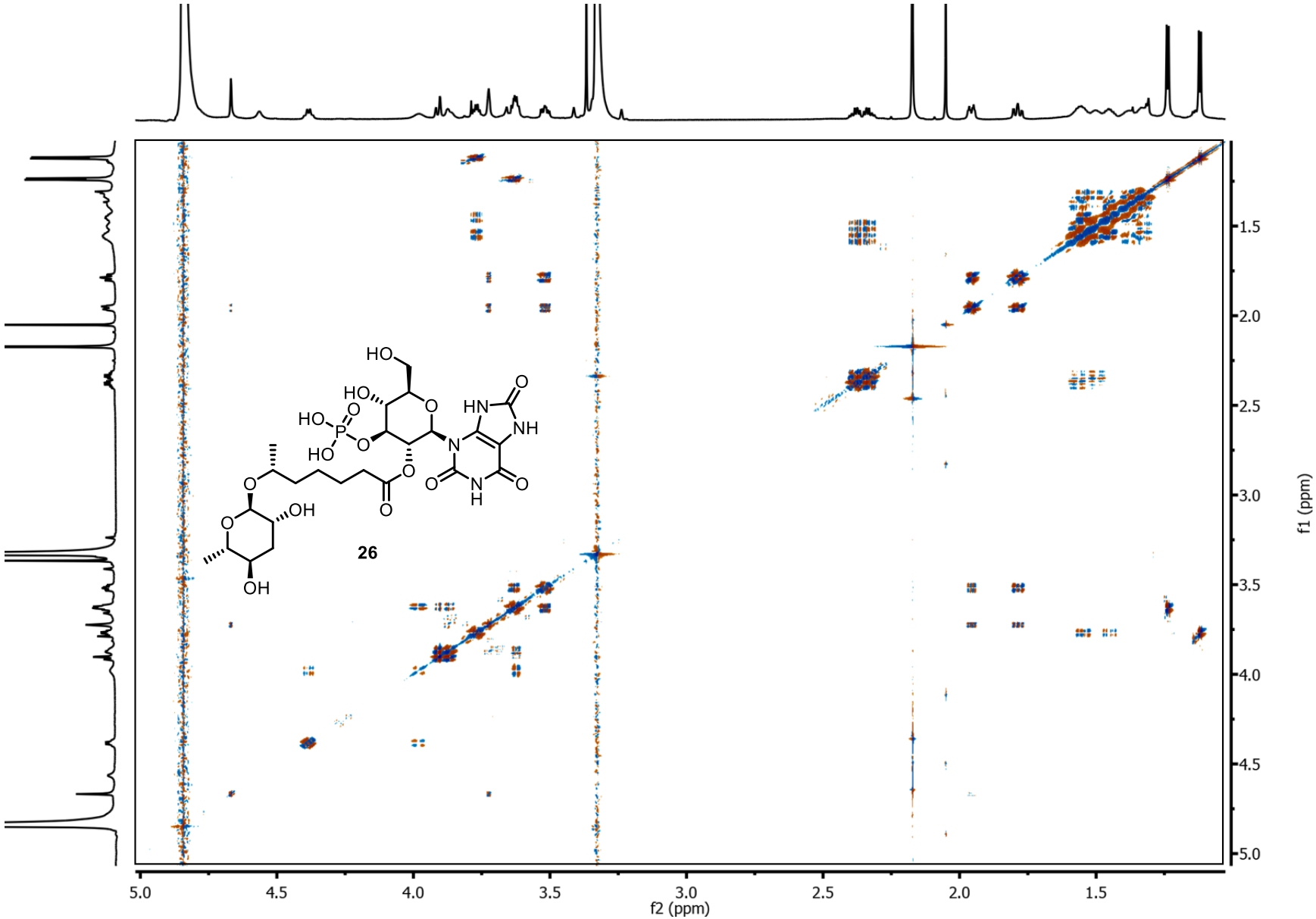
^{13}C NMR spectrum (201 MHz) of **26** in methanol-d_4. Processed using strong apodization (gf = 16, MNOVA) and baseline corrected using the Whittaker Smoother (MNOVA).



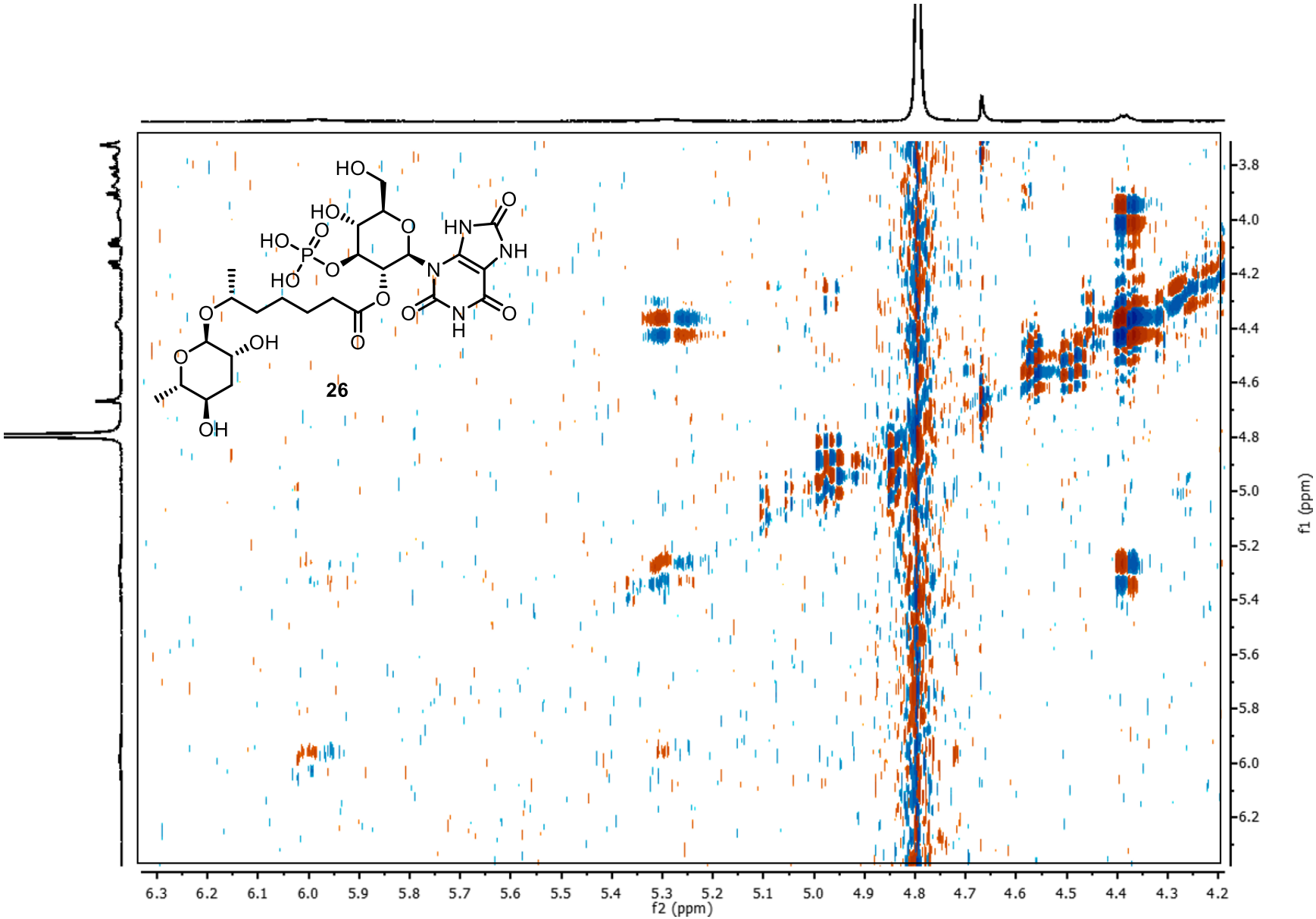
HSQC spectrum (800 MHz) of **26** in methanol-d_4.



HMBC spectrum (800 MHz) of **26** in methanol-d_4.



dqfCOSY spectrum (800 MHz) of **26** in methanol-d_4.



Section of dqfCOSY spectrum (800 MHz) of **26** in methanol-d_4 showing 2′-*O* acyl, 3′-*O* phospho substitution.